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TABLE OF CONTENTS

CHAI, C. K., Analysis of quantitative inheritance of body size in mice. I. Hybridization and maternal influence 157

CHAI, C. K., Analysis of quantitative inheritance of body size in mice. II. Gene action and segregation 165

LAIDLAW, HARRY H., JR., F. PIMENTEL GOMES, and WARWICK E. KERR, Estimation of the number of lethal alleles in a panmitic population of *Apis mellifera* L. 179

SANDLER, L., and E. NOVITSKI, Evidence for genetic homology between chromosomes I and IV in *Drosophila melanogaster*, with a proposed explanation for the crowding effect in triploids 189

NOVITSKI, E., and L. SANDLER, Further notes on the nature of non-random disjunction in *Drosophila melanogaster* 194

BAILEY, DONALD W., A comparison of genetic and environmental influences on the shape of the axis in mice 207

AMBELLAN, ELIZABETH, and TIMOTHY PROUT, Interactions between some recessive lethal and visible mutations of the fourth chromosome of *Drosophila melanogaster* 223

PITTENGER, THAD H., and K. C. ATWOOD, Stability of nuclear proportions during growth of Neurospora heterokaryons 227

COOPER, KENNETH W., Phenotypic effects of Y chromosome hyperploidy in *Drosophila melanogaster*, and their relation to variegation 242

SHIFRISS, OVED, Sex instability in Ricinus 265

SCHULER, JOSEF F., and G. F. SPRAGUE, Natural mutations in inbred lines of maize and their heterotic effect. II. Comparisons of mother line vs. mutant when outcrossed to unrelated inbreds 281

CALE, GLADSTONE H., JR., and JOHN W. GOWEN, Heterosis in the honey bee (*Apis mellifera* L.) 292

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ANALYSIS OF QUANTITATIVE INHERITANCE OF BODY SIZE IN MICE. I. HYBRIDIZATION AND MATERNAL INFLUENCE[1]

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Received April 14, 1955

ABOUT 40 years ago in his extensive experiments with hooded rats, CASTLE (1916) demonstrated that by selective breeding the average grade of coloration could be carried far beyond the limits of variation of the foundation stock. He was among the first to prove that selection could produce permanent change in quantitative characters. CASTLE's results on quantitative variation have stimulated investigation in different fields of genetics which continue to the present day. Contributions concerning gene action as well as estimation of number of factors affecting quantitative characters have been derived from both Drosophila and plant experiments.

A few extensive studies with laboratory mammals have achieved remarkable success, in spite of the difficulties in obtaining a large amount of well controlled genetic material, the longer periods of breeding required and the complicated environmental effects encountered. In three crosses between races of rabbits having different mean body weight, CASTLE (1921) estimated the minimum number of segregating factors as 3, 14 and 22. WRIGHT (1934) found a minimum of 4 segregating units in a cross between a 4-toed and 3-toed strain of guinea pigs. GREEN (1950) reported at least 3 factors involved in a cross between two strains of mice with different number of lumbar vertebrae. All these estimates were minimum and based on rather restricted assumptions.

Selection experiments for large and small body size in mice have been successful. Both GOODALE (1941) and MACARTHUR (1944) have separately established relatively large-bodied strains of mice by continuous selection. MACARTHUR also established a small-bodied strain. From mouse populations (originated from MACARTHUR's stock) of large, medium and small body size, WARWICK and LEWIS (1953) demonstrated that selection for large and small 60-day weight through five generations was effective, both in populations in the process of inbreeding and in outbred. The same authors (1954) reported in a cross-breeding experiment that the body size of most of the progenies were intermediate between the parental types. FALCONER (1952) showed again the effectiveness of selection by starting with entirely different foundation stocks. The latter has estimated at least 19 pairs of genes involved in his experiment and at least 54 in MACARTHUR's. He also concluded that the genes acted on a geometric scheme.

The current study is based on crosses between mouse strains with different body sizes, which ranged from extremely low to extremely high. Only results based on the data of pure strains and their F_1 hybrids are given in this part. Analysis based on the data of the F_2 and the backcrosses is still incomplete and will be given later. The

[1] This investigation was supported by a research grant C-1074(C4) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

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object of the experiment was to study the relative magnitude of genetic and maternal influence and to locate any effects of hybridization on body size which may occur in mice.

MATERIALS AND METHODS

Five strains of mice were used in this investigation, namely LG (Large, GOODALE), BALB/cAnd, C57BR/cd, DBA/1 and SM (Small, MACARTHUR), and the four F_1 hybrids from the crosses between LG and each of the other four strains. The following symbols were used to designate the different parental strains and F_1 hybrids throughout the paper.

Parental strains	F_1 hybrids
L—LG	CL—F_1 from C♀ × L♂
C—BALB/cAnd	LC—F_1 from L♀ × C♂
B—C57BR/cd	and similarly for the other F_1
D—DBA/1	hybrids.
S—SM	

The mice in this experiment were bred at or shortly after 60 days of age. Each breeding pair was kept in a separate pen, and was checked for newborn offspring every morning. All young were weaned when they were 28 days old. Female and male offspring were kept in separate pens with a maximum of six in each pen. Body weight was taken at birth, weaning, and 60 days of age. It has been shown that the growth curve of a mouse has reached a plateau after 60 days of age (BUTLER 1950). Variations caused by environmental factors, i.e. changes in health and fat deposition are likely to occur thereafter. Hence, for this study the 60-day body weight is considered as a reliable estimate of adult body size. The matings were started in September 1953 and were ended in June 1954. Both pure bred and cross bred matings were kept parallel in order to make concurrent observations. The 725 mice used in this study include 259 pure strain and 466 F_1 hydrids. The number of animals involved in each strain and cross is given in table 1. The relative body sizes are shown in plate 1.

As far as the history of these pure strains is concerned, strain BALB/cAnd, C57BR/cd and DBA/1 are long established inbred strains and need no description.

TABLE 1

Means ($\bar{x}$), *standard deviations* (s), *coefficients of variation* ($s/\bar{x}$) *of body weight in the pure strains and the* F_1*'s, mid-parent means, and the potence ratio of the* F_1*'s*

Pure strains					F_1 hybrids						Potence
Genotype	N	$\bar{x}$	s	$s/\bar{x}$	Genotype	N	$\bar{x}$	$\bar{x}_a$	$\bar{x}_g$	s	$s/\bar{x}$	
S	41	13.6	1.4	10.3%	LS + SL	161	25.8	25.5	22.6	2.4	9.3%	.028
D	31	18.6	1.7	8.4%	LD + DL	93	28.3	28.0	26.3	2.9	10.1%	.038
B	34	20.5	1.3	6.5%	LB + BL	102	29.4	29.0	27.7	1.5	5.2%	.055
C	88	21.0	1.5	7.2%	LC + CL	110	29.3	29.2	28.0	1.7	5.8%	.021
L	65	37.4	3.3	8.9%								

$\bar{x}_a$ — mid-parent (arithmetic).
$\bar{x}_g$ — mid-parent (geometric).

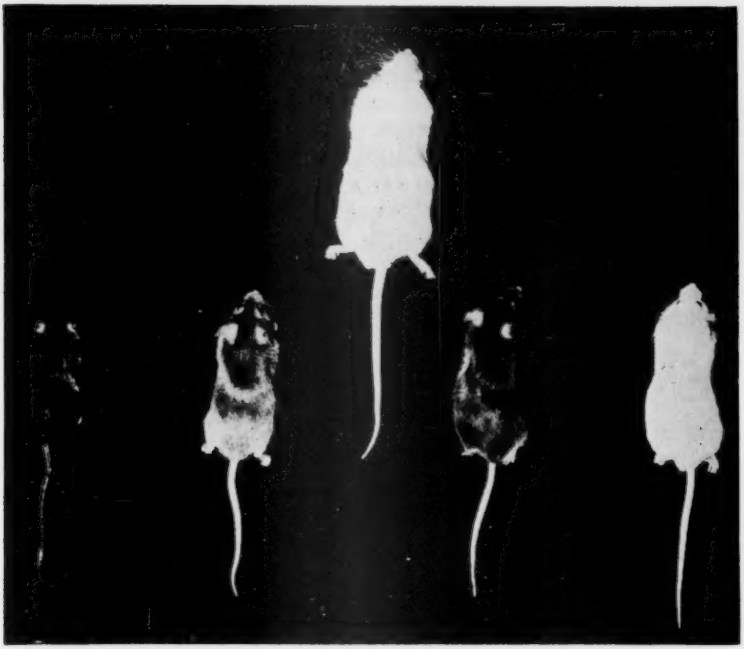

PLATE 1.—Male mice of the five pure strains. One on the top is LG. In the second row from left to right are SM, DBA/1, C57BR/cd and BALB/c.

However, the Large and Small strains are relatively new and up to the start of this experiment have passed through several methods of breeding. The Large strain was originated by DR. H. D. GOODALE before 1937. He selected many generations for large body size. Detailed information is not available concerning the size of the stock subjected to selection and the number of generations involved. In 1948 some of these Large mice were sent to DR. M. N. RUNNER of the Jackson Laboratory. They were sib-bred for four or five generations and then in an attempt to increase vigor inter-family matings were made. Sib breeding was resumed for three generations before the onset of this experiment. Because of lack of detailed information before they entered the Jackson Laboratory it is impossible to calculate the coefficient of inbreeding. It is probable that through the repeated processes of interbreeding, crossing between families and inbreeding a considerable amount of heterozygosity may well have been reduced in these animals.

The Small strain, originated by MACARTHUR by selection for small body size, had undergone 26 generations of selection at the time of its arrival at the Jackson Laboratory. Thereafter, under DR. RUNNER, it has been continuously inbred by brother-sister mating. The Small mice used in this experiment had been brother-sister bred for at least 12 generations. They were all descendents from a single pair of ancestors

at the tenth generation of brother-sister mating. The coefficient of inbreeding was estimated about 1–3% increase each generation under MACARTHUR (1944). Therefore, there is little doubt that the value of inbreeding coefficient of the Small mice was brought not far from 100% at the time when they were assigned to the experiment.

The coat color of the Large mice is albino, and their genotype is *aa cc*. They are relatively long bodied and long tailed and not very fat. The hair of the Large mice is coarse, long and loosely attached. They are susceptible to external parasites, and infections of the skin of the ears and face are frequent. They are tame and slow in action. The coat color of the Small mice is black or agouti, since the strain is maintained by forced heterozygosity ($aa \times Aa$) at the agouti locus. Small mice are quite active but not timid.

RESULTS AND ANALYSIS

Genetic considerations

The 60-day body weight of mice in each strain and their F_1 hybrids are given in table 1. The average mid-parental body weight for each F_1 hybrid has been calculated on the geometric as well as the arithmetic scale and is given in the same table. The potence ratio was computed for each cross according to MATHER (1949) by applying the following formula:

$$\frac{F_1 - P_m}{|P - P_m|}$$

where P_m refers to mid-parent and P can be either parent. The potence ratio might be interpreted as an estimate of the dominance value for the F_1 involving the two parents under the dubious assumption that loci effects are isodirectional and the dominance increments of genes have the same sign and are constant.

It has been found that there was significant difference in body weight between male and female mice. In order to have equal representation of weights for males and females, the mean body weight of each genotype was obtained by averaging the mean of the males with that of the females. The same procedure was employed for computing the standard deviations. According to the distribution of the means of the pure strains, the Small and Large strains are at the two extremes, while the other strains are intermediate in range. The F_1 means have the same sequence as their parent strains except for the means of LB + BL and LC + CL which are very close in magnitude. This is probably due to the fact that there is no significant difference in body size between the two parent strains, B and C.

A comparison of the F_1 means with their mid-parental means on the arithmetical scale can be made in the graph illustrated in figure 1. In this graph, the intersects of the lines joining the Large and each of the other strains with the center line are the arithmetric means of the mid-parents. The points on the center line are the observed means of the F_1 hybrids, and are slightly above their respective mid-parental values. These results may be interpreted to mean that the net effect of favoring large body

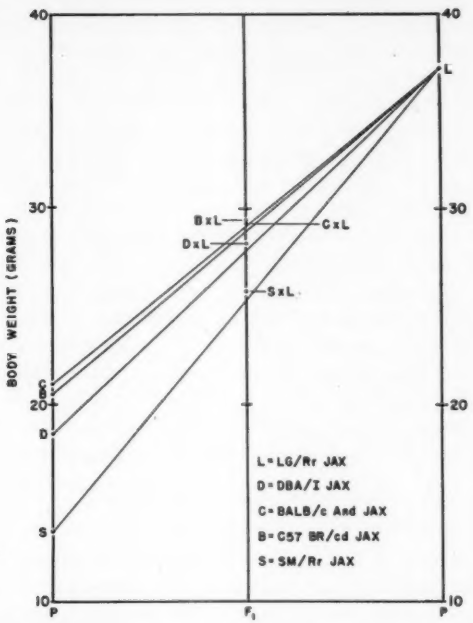

FIGURE 1.—A graph illustrating body weight of the F_1 hybrid in relation to the mid-parent. The intersects of the lines LS, LD, LB and LC with the center line are the mid-parent values, and the points on the center line are the observed means of each of the F_1 hybrids.

size in the Large strain is slightly dominant over the genes in the other strains. This finding may also be explained as due to hybrid vigor in favor of large size.

It has been demonstrated that in plants (red pepper, KHAMBANONDA 1950) (tomato, POWERS 1941) and animals (mouse, MACARTHUR 1944 and FALCONER 1953) that genes involved in quantitative characters are more likely to act geometrically than arithmetically. Table 1 shows that each F_1 hybrid is above its mid-parental value on either an arithmetic or a geometric scale, with the departure of the F_1 means from the geometric means of the parents further than that from the arithmetric means of the parents. However, the standard deviations showed that the strains of larger size had larger standard deviations. Furthermore, all the standard deviations of the F_1 hybrids, especially in the crosses of L with S and D, were larger than those of their respective parental strains, C, B, D and S. This is true in spite of the greater expected constancy (due to homeostasis) of the F_1 hybrids as compared to that of the pure parental strains. When correlating standard deviations with their means there appear quite large random errors perhaps due to the small sample size. Nevertheless, the values fell approximately in line on a geometric scheme, which was considered as the appropriate scale in the previously cited studies.

The potence ratios, although not identical between different crosses, are probably not significantly different. The average potence for all is 3.7%.

Variation within any of the isogenic populations or their F_1 hybrids is generally regarded as environmental. ROBERTSON and REEVE (1952) demonstrated that in Drosophila the variation in the F_1's was less than in the parental strains. The authors explained this as due to the better adaptability of the F_1 than that of the homozygous individuals. It ought to be mentioned that some environmental factors, causing variation in mammals are quite different from those in Drosophila. Factors such as litter size, age of the mother, uterine environment and nursing are important in mammals and almost non-existent in insects. In mice, GRÜNEBERG (1954) suggested that in some polygenic characters the homozygous state tends to be less stable developmentally than the heterozygous one. MCLAREN and MICHIE (1954) showed F_1 hybrid mice were less variable in response to bioassay than the inbred lines. Table 1 gives the coefficient of variation in body weight for each F_1 hybrid and each pure strain. Although they vary considerably in the F_1's and in the pure strains, the average coefficient of variation of the F_1's appears smaller than that of the pure strains. This seems to be in accord with the findings of the other different investigators.

Maternal influence considerations

As each F_1 hybrid was produced by a mother from one of two different strains, a comparison can be made of the magnitudes of the influence of genetic constitution and maternal effect upon body size. In table 2, the average body weights of the males and the females are given for each F_1 hybrid. The data show that sex, genotype and maternal influence play important roles in determining body size in mice. Analysis of variance was employed in order to find the relative importance of each. If all sources of variation were considered and were carried in one analysis of variance, a three-way classification would be required. In addition, since there would be unequal numbers of animals in the subgroups, the analysis would be a complex nonorthogonal case. However, as maternal and genotypic effects are the essential factors to be considered, the analysis will be made much simpler by separating the data into two parts according to sex. Hence, the analysis has been carried out following the method of disproportionate subclass numbers (outlined by SNEDECOR 1946) in a two-way classification involving maternal parent and genotype only. The complete results of the analysis of variance will not be given to save space. Only the absolute values and the

TABLE 2

Mean body weight of males, females and their average of each F_1 hybrid with different maternal parent

Mothers other than L				L mothers			
Genotype	Males	Females	Average	Genotype	Males	Females	Average
SL	26.8±2.3	22.1±1.7	24.5±2.0	LS	29.1±2.9	25.2±2.9	27.2±2.9
DL	28.8±3.4	22.8±3.5	25.8±3.4	LD	34.1±2.5	27.6±2.3	30.9±2.4
BL	31.2±2.1	25.4±1.0	28.3±1.5	LB	34.0±2.5	27.1±1.1	30.6±1.6
CL	31.4±1.5	25.5±1.4	28.4±1.5	LC	33.5±2.4	26.9±1.7	30.2±2.0

TABLE 3

Sources of variance in body weight of F_1 hybrid females and males attributed to maternal, genetic, maternal × genetic and individual

Sources of variation	F_1 females			F_1 males			% of total variance
	Degrees of freedom	Mean squares	Variance	Degrees of freedom	Mean squares	Variance	
Maternal	1	417.9**	2.6	1	505.8**	4.2	26.3
Genetic	3	133.2**	1.5	3	320.5**	5.1	23.2
G × M	3	27.6**	0.7	3	26.3**	0.7	5.6
Individual	224	4.7	4.7	218	6.7	6.7	44.9

** $P < .01$.

percentages of the variances attributed to each component, maternal, genetic, maternal × genetic and individual sources, are given in table 3.

The maternal and genetic effects are both significant with probability below 1%. The source of interaction between genetic and maternal is significant with probability at a level about 1%. The variances attributed to each source take the following sequence in regard to magnitude:

Individual > Maternal > Genetic > Maternal × Genetic

The variance attributed to individual is due to environment other than maternal. It is interesting to see that when the sexes are averaged the maternal influence contributes more than one quarter of the total variation, slightly more than does the genetic constitution.

SUMMARY

A scheme for cross breeding an extremely large bodied strain of mouse with four other strains ranging from a medium to extremely small body size was carried out.

The mean body weight of each F_1 hybrid was shown to be slightly above the arithmetic mean of the mid-parent, and more above the geometric mean of the mid-parent. The calculated potence ratio (3.7%) was very low. In general, the standard deviation of both the pure strains and F_1 hybrids tended to be proportional to the means. Therefore multiplicative gene action is suggested.

The maternal influence on body size in the F_1 hybrids was estimated to contribute more than one quarter of the total variation, a larger source of variation than was the genetic constitution of the hybrids.

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ANALYSIS OF QUANTITATIVE INHERITANCE OF BODY SIZE IN MICE. II. GENE ACTION AND SEGREGATION[1]

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FISHER (1918), WRIGHT (1935) and KEMPTHORNE (1954) showed that variation and correlation between relatives ascribable to additive effects, dominance and interaction of the genes can be partitioned. Methods of estimation of the effective number of factors have been given by FISHER, IMMER and TEDIN (1932), WRIGHT (1934, 1950), CASTLE (1921) and MATHER (1949).

In mice, BUTLER (1952) has made five crosses between strains different in body size. He found that the F_1 and F_2 means were half way between those of the parents. backcross means were half way between that of the F_1 hybrid and that of the pure-strain parent. As expected, the variances of the parental strains and the F_1 hybrids were similar; however, the variance of the F_2 was no larger than that of the F_1.

In the present study the genetic component of the quantitative variability of body size in mice has been analyzed. Among the matters that have been considered were number of loci or at least effectively independent blocks of loci, the degree of dominance among the loci, the distribution of gene effects, and the amount of environmental contributions to the total variation.

BREEDING PLANS

The Large and Small strains of mice were used as the parental stocks. Their historical developments have been previously reported. There were 12 cross-bred types used: two F_1, two F_2 and eight first-backcross generations. The type and number of matings, average litter sizes and designations for each of the different types of matings are given in table 1. Throughout this paper "subgroup" refers to all the females or males under each different type of mating and "genotypic group" refers to all the individuals within each pure strain or hybrid, i.e. P_L, P_S, F_1, F_2, B_L and B_S.

Maternal influence within each hybrid group was balanced by making reciprocal matings between the two parental genotypes. For instance the F_1 hybrids were produced by two different types of matings, L $\times$ S and S $\times$ L. Other hybrids were similarly produced. Maternal effects will still exist between crosses, and will be considered at length below. Inbreeding was avoided as much as possible in the production of the F_2 and backcross mice. There were no matings closer than second cousins or single first cousins. Therefore the effects of inbreeding were trivial and no correction is needed.

Prolificness varied between mice of different genetic constitutions. The hybrid mouse was a better reproducer than the pure-strain mouse. This is evidenced by large litters in the F_2 generation and in those backcross mice with the F_1 hybrid as the

[1] This investigation was supported by a research grant C-1074(C4) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

TABLE 1

The type and total number of matings, with the average size of litters in each type of mating

To produce	Type of mating	Total no. matings	No. of litters	Lit. avg.	Size range
Large	L × L	9	22	6.0	1–10
Small	S × S	7	19	4.5	2–7
F_1	L × S	6	14	4.6	1–9
	S × L	10	24	5.5	1–8
F_2	$(L \times S)^2$	4	17	10.9	4–16
	$(S \times L)^2$	4	16	11.6	9–15
B_L	SL × L	2	9	5.8	2–10
	LS × L	2	9	10.2	8–14
	L × SL	2	5	8.0	5–12
	L × LS	2	5	8.0	4–10
B_S	SL × S	1	4	9.5	8–10
	LS × S	2	5	8.5	2–12
	S × SL	4	10	5.7	3–8
	S × LS	3	11	5.2	3–9

maternal parent (table 1). The F_1 mothers also had very short intervals between litters. Practically all the F_2 and some of the backcross animals came from early litters of their parents, while the F_1 and the pure-strain animals studied were produced throughout the life history of their parents. In order to give the young equal suckling opportunity, a maximum of 8 young were kept in a litter.

RESULTS AND ANALYSIS

A total of 801 mice were included in this study. The number of animals, means, variances, and coefficients of variation of body weight for each pure strain and each subgroup are given in table 2. The distributions of body weight for each genotypic group are illustrated in figure 1.

The average body weight of males was greater than that of females in each subgroup of mice. In order to circumvent the complications of sex differences and unequal sex ratios, a subgroup mean for 60-day weight was taken to be the unweighted average of the mean weights of the male and female mice in the subgroup. Similarly, since the number of mice in each subgroup varied, the mean body weight of each hybrid group has been computed by averaging all the unweighted subgroup means. The same procedure has been employed for combining the variances and the coefficients of variation.

Before presenting the results of the biometrical analysis, it seems necessary to consider the effects of litter size, age of the mother and maternal influence. We shall first examine the maternal influence. As each hybrid was produced by mothers of different strains, maternal effect between strains, if different, would contribute unequally to the means of the different hybrids. It appeared that F_1 hybrid mothers had more augmenting influence to the young than purebred mothers and similarly the Large mother had more augmenting influence to the young than the Small mother.

TABLE 2

The means ($\bar{x}$), variances (s^2) and coefficients of variation (C) of 60-day body weight in each subgroup of mice

	Genotype	No. of animals	$\bar{x}$	s_l^{2*}	s_i^{2**}	s_t^{2***}	C_l^*	C_i^{**}	C_t^{***}
P_L	(L × L) ♀	20	33.23	8.36	1.86	10.22	.0870	.0411	.0962
	(L × L) ♂	45	41.48	8.36	5.97	14.33	.0697	.0588	.0912
P_S	(S × S) ♀	20	12.84	1.35	0.26	1.61	.0906	.0397	.0988
	(S × S) ♂	21	14.37	3.05	0.63	3.68	.1223	.0553	.1335
	Total	106							
F_1	(L × S) ♀	30	25.23	8.18	.98	9.16	.1134	.0392	.1200
	(L × S) ♂	35	29.08	7.37	1.92	9.29	.0934	.0476	.1048
	(S × L) ♀	53	22.13	1.98	.96	2.94	.0636	.0442	.0775
	(S × L) ♂	43	26.80	4.33	1.24	5.57	.0776	.0415	.0881
	Total	161							
F_2	(S × L)^2 ♀	55	25.00	0	5.66	5.56	0	.0952	.0952
	(S × L)^2 ♂	58	30.72	1.63	9.89	11.52	.0415	.1024	.1105
	(L × S)^2 ♀	52	24.57	1.73	5.26	6.99	.0539	.0937	.1076
	(L × S)^2 ♂	51	28.54	0	9.46	9.46	0	.1077	.1077
	Total	216							
B_L	(S × L)L ♀ (L × S)L ♀	58	29.16	2.44	3.69	6.43	.0535	.0659	.0849
	(S × L)L ♂ (L × S)L ♂	56	36.25	5.52	5.48	11.0	.0648	.0646	.0915
	L(S × L) ♀ L(L × S) ♀	26	28.41	0	7.73	7.73	0	.0979	.0979
	L(S × L) ♂ L(L × S) ♂	24	34.20	8.63	9.88	18.51	.0858	.0919	.1258
	Total	164							
B_S	(S × L)S ♀ (L × S)S ♀	23	19.68	8.83	4.07	12.90	.1503	.1023	.1825
	(S × L)S ♂ (L × S)S ♂	36	22.60	5.31	6.82	12.13	.1023	.1156	.1541
	S(L × S) ♀ S(S × L) ♀	41	18.10	.97	2.64	3.61	.0545	.0898	.1050
	S(S × L) ♂ S(L × S) ♂	54	21.28	1.88	3.27	5.15	.0646	.0852	.1066
	Total	154							

* l = between litters. ** i = within litters. *** t = total

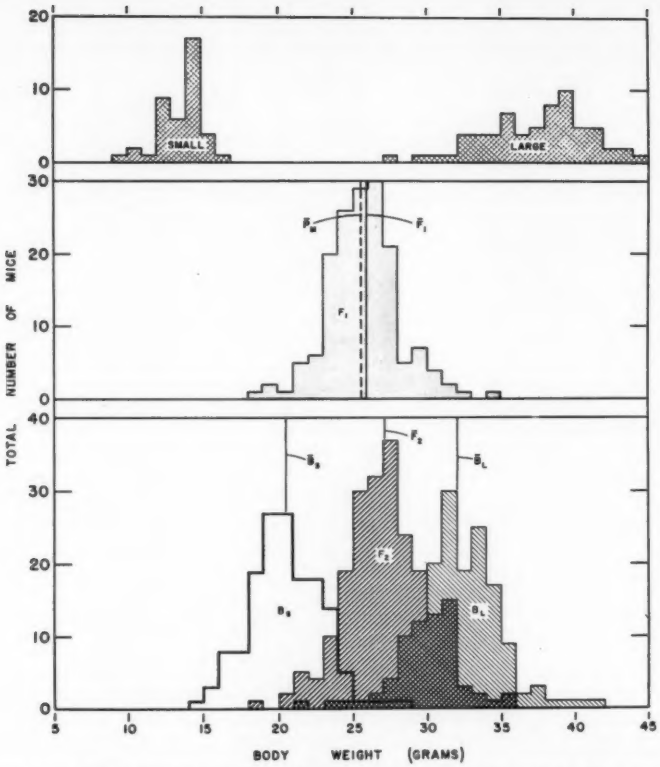

FIGURE 1.—A graph showing the distributions of 60-day mouse body weight of the pure strains, Large and Small, and the hybrids, F_1, F_2, B_L and B_S. It was constructed by superposing all subgroups within each genotype so that all the subgroup means are lying on their general mean.

The average sizes of litters from matings producing pure-strain and F_1 hybrid offspring were similar, i.e. 4.5 to 6.0 (table 1). The average sizes of litters in matings producing the F_2 generation were 10.9 and 11.6. The sizes of litters in the backcross generations were intermediate between the two extremes, 5.2 to 10.2. Since body weight at 60 days of age may be influenced by size of litter the observed mean body weight in the F_1 hybrids would be overestimations, and the mean body weights in the F_2 would be underestimations. Effect of parental age may tend to offset the bias due to litter size since F_2 mice were produced during the first part of the productive age of their parents and the pure strains and F_1 mice were produced throughout the life history of their parents. The method of computing multiple regression coefficients has been often employed to correct for such effects (EATON 1954). However, it could not be satisfactorily applied to the present data because the sample sizes were too small to consider each subgroup separately. On the other hand, the differences be-

tween the subgroups in the mean body weight and pattern of reproduction of their parents would make the computed coefficients of the pooled sample meaningless. It is generally considered that the preweaning environment of either first litter or late litters in mice is less favorable than that of the middle litters. Therefore, in the pure-strains and F_1 hybrids, mice of the late litters may have the tendency to reduce the mean body weight and, in turn, result in an underestimation. Nevertheless, errors in estimation of body weight introduced from such sources are difficult to evaluate.

With regard to the effects of litter size, mother's age and certain maternal influences upon body-weight variances in the different subgroups, it is thought that variations from these sources exist mainly between litters rather than within litters. By removing the between-litter variation from the total variation, effects from such sources can be eliminated. Therefore, the method of analysis of variance has been employed to separate the variations between litters and within litters. The variance due to litter, s_l^2, and the variance due to individuals within litter, s_i^2, as well as the corresponding coefficients of variation, C_l, C_i, for all the subgroups have been computed (table 2).

The variances and coefficients of variation due to litter vary among subgroups within the Small strain, F_1 and backcross generations. If each genotypic group as a whole is considered, the F_2 hybrids give the lowest values. From the analysis of variance it has also been shown that the variation due to litter in the F_2 hybrids was nonsignificant while that in the parental strains and the F_1 hybrid was significant ($P < .01$). As the 8 different backcross subgroups show two are nonsignificant, two are significant at the 5% probability level and four are significant at the 1% probability level. Although some sampling errors due to smaller number of individuals contained in certain subgroups may be unduly large, the results seem, in general, to indicate the lack of consistency of environments provided by the mothers and the differences in litter sizes between the pure strains and the F_1 hybrids. As was expected, variation from such sources was larger in those subgroups whose parent, particularly the maternal one, belonged to a pure strain. Variations due to such unevenly distributed environmental sources have been found to differ among genotypic groups or their subgroups. These variations must be removed in order to arrive at a genetic analysis of these genotypic groups.

Having removed variations caused by factors that were unequally distributed among the parental strains and hybrids, e.g. variation due to litter, the residual within-litter variation within the different genotypic groups would measure the amount of variation attributable to different genotypes under similar environmental influences. This may be expressed by the within-litter variance, s_i^2. It is noticed that the values of the within-litter variances of the subgroups within the same genotype are more similar than the average values between genotypes.

WRIGHT (1952) has given the formulae for estimating the mean and variance on the logarithmic scale from the actual mean and coefficient of variation.

$$\overline{log_{10} x} = log_{10} \bar{x} - \tfrac{1}{2} log_{10} (1 + C^2)$$
$$s^2_{log_{10} x} = .43431\ log_{10} (1 + C^2)$$

By applying the above formulae the logarithmic values of both means and variances have been obtained (table 3).

TABLE 3

Means and variance of body weight of mice in each genotype on logarithmic and actual scales

Genotype	$\bar{x}$ obs.	$\bar{x}$ corr.	$\bar{x}$ theo.	s^2	$\overline{\log x}$ obs.	$\overline{\log x}$ corr.	$\overline{\log x}$ theo.	$s^2_{\log x}$	Correction factors	
P_L	37.35	35.98		3.92	1.56778	1.54121		.00049	$-x$	0
P_S	13.61	13.61		0.46	1.13004	1.13004		.00044	0	0
P_M	25.48	24.80		2.18	1.34891	1.23562		.00047	$-\frac{1}{2}x$	0
F_1	25.82	25.01		1.28	1.40754	1.39425		.00036	$-\frac{1}{2}x$	0
F_2	27.10	25.10	24.95	7.57	1.43175	1.39499	1.36493	.00191	$-\frac{1}{2}x$	$-y$
B_L	31.59	30.77	30.50	6.70	1.50067	1.46900	1.46773	.00125	$-\frac{1}{2}x$	$-\frac{1}{2}y$
B_S	19.72	19.36	19.31	4.21	1.30419	1.28581	1.26214	.00184	$-\frac{1}{2}x$	$-\frac{1}{2}y$

x = correction for large dam. y = correction for crossbred vs. inbred dam.

The two sources of maternal effect, the differences between the Large dam and Small dam and the differences between the crossbred and inbred dams, need to be corrected before considering the means of the different genotypic groups. A generalized correction factor of each for the means is derived according to WRIGHT (personal communication):

$$x = (L \times S) - (S \times L)$$
$$-x = (F_1 \times L) - (L \times F_1) - (F_1 \times S) + (S \times F_1)$$
$$2y = (F_1 \times L) - (L \times F_1) + (F_1 \times S) - (S \times F_1)$$

where x is the correction factor which converts the Large dam to the Small dam basis and y that which converts the crossbred dam to the inbred dam basis. The following values were obtained according to the above equations:

$$x = 1.37 \text{ (actual scale)}, .02657 \text{ (log scale)}$$
$$y = 1.42 \text{ (actual scale)}, .02347 \text{ (log scale)}$$

The necessary corrections are listed in the last column of table 3. The observed, corrected and theoretical means, and the within litter variances in the different genotypic groups are given in the same table. The mean of the F_1 hybrids is close to the midparent value and the means of the backcross fall between the mean of the F_1 and that of their respective parental strain. It is observed that the corrected means are in better agreement with the theoretical means on the actual scale than on the logarithmic scale. The variance of the F_2 hybrids gives the highest value, the backcross hybrids the intermediate values, and the F_1 and the parental strains the lowest values. These orders of both means and variances are as expected.

TESTS OF THE SCALE

The choice of an adequate scale is important for the analysis of quantitative variability. The best scale for the purpose of such an analysis is one on which the effects of factors (genetic and environmental) are as nearly additive as possible. The logarithms of measurements of organisms are often used in statistical studies based on the hypothesis that the growth factors tend to contribute constant percentage increments (POWERS 1942) or to act on a geometric scheme. It appears to be necessary to

compare the fitness of the two different scales, actual gram weight and logarithmic gram weight, to the present data.

On an adequate scale for the theoretical means for each of the hybrids, the following equations according to WRIGHT (1952) will hold:

$$\bar{F}_2 = \tfrac{1}{2}(\bar{P}_M + \bar{F}_1)$$
$$\bar{B}_L = \tfrac{1}{2}(\bar{P}_L + \bar{F}_1)$$
$$\bar{B}_S = \tfrac{1}{2}(\bar{P}_S + \bar{F}_1)$$

where the subscript M denotes mid-parent. Several statistics which may be used for testing the adequacy of a scale of measurement with respect to additivity when the two pure strains, F_1 and F_2 and the backcrosses are available have been given by MATHER (1949):

$$A = 2\bar{B}_L - \bar{P}_L - \bar{F}_1 \qquad s_A^2 = (4s_{B_L}^2 + s_{F_L}^2 + s_{F_1}^2)$$
$$B = 2\bar{B}_S - \bar{P}_S - \bar{F}_1 \qquad s_B^2 = (4s_{B_S}^2 + s_{F_S}^2 + s_{F_1}^2)$$
$$C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_L - \bar{P}_S \qquad s_C^2 = (16s_{F_2}^2 + 4s_{F_1}^2 + s_{P_L}^2 + s_{P_S}^2)$$

Where A, B, and C are quantities denoting the discrepancies of the observed means according to the theoretical equations and S_A, S_B, and S_C are the respective standard errors. Tests of significance may be carried by the customary methods. If the scale is adequate these quantities will each equal zero within the limits of their standard errors. The results of the three tests for the adequacy of the actual scale and the logarithm scale are:

	$A \pm s_A$	$B \pm s_B$	$C \pm s_C$
Actual weight	.55 ± 1.59	.10 ± 1.20	.79 ± 1.97
Logarithmic weight	.0025 ± .0192	.0473 ± .0266	.1202 ± .0322

The above results seem to indicate that the actual scale is superior than the logarithmic scale. However, this test is merely based on the comparison between the

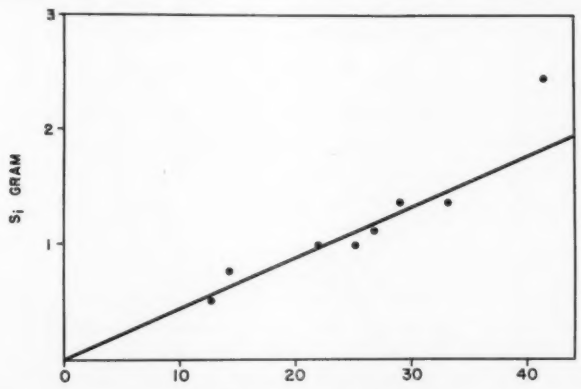

FIGURE 2a.—A plot of the standard deviation within litter against the mean body weight in each subgroup of the Large, Small and F_1 hybrid. It shows that the standard deviation increases proportionally to the mean.

means of the different groups of mice. Some tests based on the other statistics have to be considered.

One test is the comparison of standard deviations with their respective means of the genetically constant populations. If the standard deviation increases with increase of the mean, multiplicative gene action and/or environment genic interaction are suggested. In these data, the standard deviations of the F_1 hybrid and parental subgroups are approximately proportional to their means (fig. 2a). Consequently the coefficients of variation are about the same size allowing for random deviation (fig. 2b).

One could also use the standard deviations and the means of the backcrosses, both B_L and B_S, if there were no dominance. Although this test was not as indicative

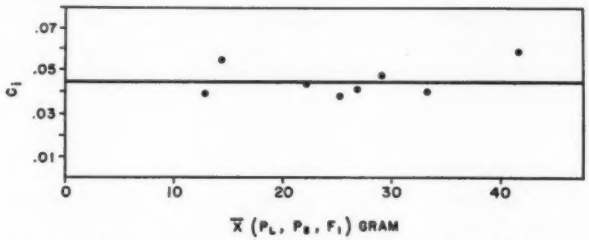

FIGURE 2b.—A plot of the coefficient of variation against the mean body weight in each subgroup of the Large, Small and F_1 hybrid. It shows that the coefficients vary randomly about their mean

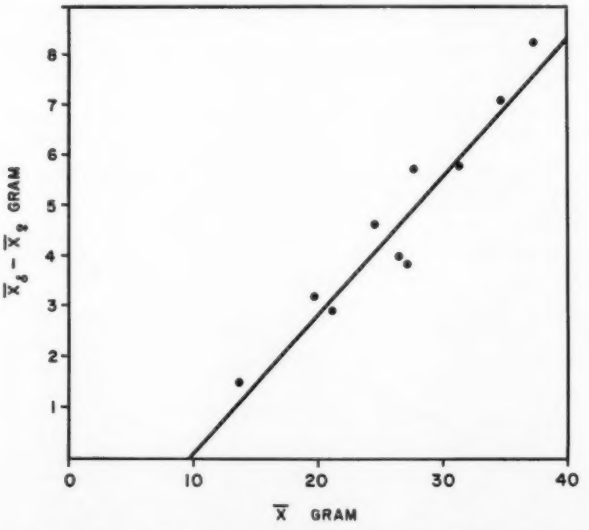

FIGURE 3a.—A plot of sex difference against the mean in gram body weight in each subgroup of the Large, Small and F_1 hybrid. The regression coefficient of sex difference on the mean in gram body weight is .28. The regression line intercepts the x-axis at about the 10 gram point.

as the one above, the standard deviations within litter were found approximately proportional to their means.

Another test is the relation of constant factors to the scale. In this case, the difference in body weight between sexes in each subgroup is unique and can be applied effectively. It was found the larger the body size the greater the sex difference in body weight; that is, the difference is proportional to the mean in each subgroup (fig. 3a). This is a third bit of evidence which suggests multiplicative gene action. From the results of the different tests and previous investigations, it is felt that, although the actual scale appeared to fit the means better, the logarithmic scale is more adequate when all evidence is considered. Therefore, in the following analyses, only the means and variances on the logarithmic scale are considered.

PARTITION OF VARIANCE AND ESTIMATION OF SEGREGATING UNITS

Several statistics concerning the various proportions of genetic and environmental variances were given by FISHER, *et al.* (1931) and WRIGHT (1952) on the assumption that non-additive interactions have been eliminated by use of an adequate scale. The following equations are based on the symbols adopted by WRIGHT.

$$\sigma_P^2 = \sigma_{F_1}^2 = \sigma_e^2$$
$$\sigma_{F_2}^2 = \sigma_g^2 + \sigma_d^2 + \sigma_e^2$$
$$\sigma_{B_1}^2 + \sigma_{B_2}^2 = \sigma_g^2 + 2\sigma_d^2 + 2\sigma_e^2$$

The genetic variance may be analyzed into an additive component, σ_g^2, due to the difference between the homozygotes of each pair of genes; and a genetic dominance component, σ_d^2, due to the difference between the heterozygote and the mid-parent of each pair of genes. The relative proportion of σ_g^2 and σ_d^2 is different between the F_2 and backcross hybrids. The environmental variance σ_e^2, can be estimated by the variance within the pure strain or the F_1 hybrid. Using the variance of only the F_1 hybrid would have the advantage of eliminating any bias due to inadequate scaling, but any error due to genetic homeostasis will be introduced. Consequently, the average of the variances of the parental strains and the F_1 hybrid may be a better estimate of σ_e^2 than one based on either parental strain or F_1 hybrid alone. On the within litter basis it was found to be .00041. Using the computed variances from the different genotypic populations, it is possible to solve the following equations simultaneously, thereby obtaining the estimates of the different genetic components.

$$\sigma_g^2 = 2\sigma_{F_2}^2 - (\sigma_{B_L}^2 + \sigma_{B_S}^2) \quad (1a) \qquad 2\sigma_g^2 = \sigma_{F2}^2 + \sigma_{B_L}^2 + \sigma_{B_S}^2 - 3\sigma_e^2 \quad (2)$$
$$\sigma_d^2 = \sigma_{F_2}^2 - (\sigma_g^2 + \sigma_e^2) \quad (1b)$$

Equations (1a) and (1b) can be used to separate dominance component from additive genetic variance, however, they magnify sampling error, whereas equation (2) incorporates the dominance component with the additive genetic variance but does not introduce a greater sampling error. The computed values of the additive genetic variance was .00189 from equation (2) and .00073 from equation (1a) and of the dominance variance was .00077 from equation (1b). The additive genetic variance .00189 estimated from equation (2) is considered to be a better estimate. The reason will be discussed later.

A formula for estimating the minimum number of segregating units, $R^2/8s_g^2$, was

TABLE 4

Estimates of the number of segregating units which distinguish the Large and Small strains with respect to body size and the interpretations of the segregating index

Segregating index	Equal effect		Geometric series		Maximum amount possible to one locus
	N	$2G_A/R$	C	$2G_A/R$	
11	11	2.03	.83	3.80	6.76

given by CASTLE (1921) (modified by WRIGHT). It is assumed that each parental strain of mouse, Large and Small, has its own set of genes having a constant effect on body size. The Small strain contributes no plus genes for body size except those that may already be present in the Large. The reciprocal situation is assumed to be equally true. Then the amount of difference in body weight, R, between the Large and Small strains could be ascribed to the blocks of genes segregating freely in the F_2 generation. Applying the computed additive genetic variance, .00189, the minimum number of segregating units was estimated at 11, using the logarithmic scale.

There are many possible interpretations with regard to the distribution of the genic effects. It may be assumed that their effects are equal among the different pairs of alleles, or their maximum effects are attributed to a single pair of alleles or thirdly that the effects of the different pairs of alleles are decreasing geometrically from a leading locus. The equations for computing the contribution from one pair of alleles according to the different interpretations has been given by WRIGHT (1952). The computed values for the effect of various combinations of pairs of alleles upon the body weight within each of the three possible interpretations have been given in table 4.

DISCUSSION

Scale

The choice of an appropriate scale is the fundamental step in the analysis of polygenic variation since the description of a body of data or a comparison of two or more bodies of data are dependent on the scale used in measurement. We cannot assume without evidence that a scale adequate to represent the variation of a character in one population under one certain kind of environment will be as adequate to represent the variation of the same character under another different environment. Nor could we assume without evidence that the same scale would suffice for the same character for different genotypes. It is certainly not possible to assign an *a priori* scale to express variation in a continuously varying character with our present knowledge of the complexities of gene action. It is essential, therefore, to devise and test some scale, which is thought possible to fit the data at hand.

There are many different types of transformations of actual measurements that are adaptable to characters with different underlying causes (WRIGHT 1952). Both MACARTHUR and FALCONER have favored the logarithmic scale for body weight in mice. Use of the logarithmic scale in this body of data can be further justified by a comparison of s^2 and $s^2_{\log x}$ between the different genotypic groups (table 3). On an adequate scale, the variances of the isogenic lines theoretically should be equal. In

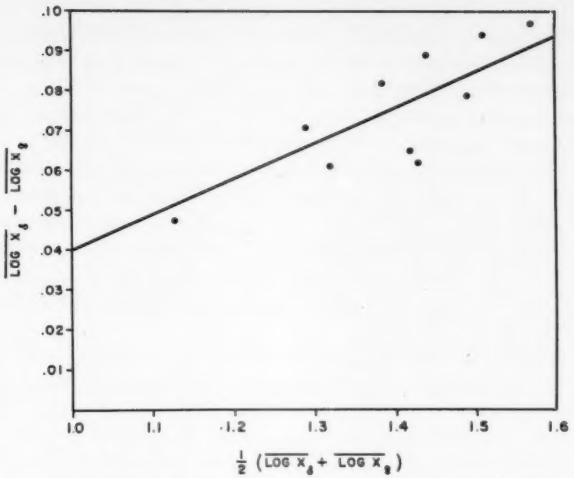

FIGURE 3b.—Illustrating, on the logarithmic scale, the sex difference in body weight in relation to the mean body weight in each subgroup of the Large, Small and F_1 hybrid. It shows that a slight regression appears still to exist.

these data on the logarithmic scale, the variances of P_L and P_S were quite similar, while on the actual scale the variance of P_L was about 9 times larger than that of the P_S. In addition, the variance of the F_1 hybrid on the logarithmic scale was shown to be closer to the parental strains than on the actual scale. The slightly lowered value of the F_1 hybrid could be attributed to genetic homeostasis of hybridization. The other noticeable difference between the two scales, is the interchange of the relative order of magnitude of the variances in the backcrosses. That is $s^2_{P_L} > s^2_{P_S}$ on the actual scale, while $s^2_{P_L} < s^2_{P_S}$ on the logarithmic scale. This does not imply necessarily that the logarithmic scale is superior to the actual scale. It indicates, however, a practical difference between the two scales when applied to the same data, and reflects the importance of choosing the appropriate scale.

In figure 3a, the regression line of sex difference on mean body weight intercepts the x-axis at about the 10 gram point, and the same regression line on the logarithmic scale is not parallel to the x-axis (fig. 3b). These results seem to indicate that log x-transformation probably does not go far enough, and log (x − 10) transformation would be more adequate.

Variance

To remove the variation between litters would undoubtedly tend to reduce some of the environmental variation which may be unequally distributed through the different populations. Sources contributing to such variation, in addition of the measurable factors, such as litter size and age of the mother, could involve any permanent or temporary irregularities of the mother such as chronic disease or short period of physiologic disturbance. The latter may retard the growth of the young,

remain undetected and hence be non-correctable, by the method of regression coefficient. The method of analysis of variance has the advantage that variation from such undetectable sources are incorporated into the between-litter variation. Only a very small portion of genetic variation is involved in the litter variation. Its effect relative to the total genetic variation could be considered practically negligible.

However, the variation between litter constituted a large proportion of the total variation. This variance has to be restored if we want to consider the over-all environmental contribution to the total variability. The average of the total variances of the subgroups in the F_1 hybrids and the pure strains may be considered as a good estimate of the over-all environmental variance. It was obtained to be .00198. Thus the environmental variation within litter contributed only about one fifth of the total environmental variation.

Whether or not dominance played an important role in this cross between mouse strains deserves consideration. According to equations (1a) and (1b), the dominance variance was estimated to be .00077 and the additive variance to be .00073. The dominance variance was even slightly higher than the additive genetic component. From the previous observations (CHAI 1956), the means of body weight of the F_1 hybrids were about the same as the means of the mid-parents. If dominance existed, it would be equally distributed between parental strains; then, and only then such a comparatively large component of dominance variance could be explained. If such a situation exists, the means of the B_L and B_S in the present study should be closer to the means of their pure parental strains than to the mean of the F_1 hybrid. On the contrary, the backcross means, although both shifted very slightly to large size, were about the same as the mid-values of the parental groups. Therefore, we very much suspect that the relatively large value of the dominance variance obtained was mainly due to magnification of sampling error. Thus both estimates of dominance variance and additive genetic variance from equations (1a) and (1b) are considered unacceptable. The main contribution to the genetic variance are most likely the genes giving only additive effects. Therefore the amount of dominance variance incorporated into the additive genetic variance (.00189) according to equation (2), if any, is assumed to be negligible.

Segregating units

The number of loci for size inheritance would be underestimated if linkage among them existed. However, it would be impossible to detect linkages without second backcross or F_2 generations. If the genes concerned in the present cross have no lethal or semi-lethal effect, linkage would not affect the frequencies with which the allelomorphs of each gene are recovered in the segregating generations. On an adequate scale, linkage can have no effect on the mean measurements of the segregating generation. It, however, shows its effect in the second degree statistics, the variance. At the present stage of our knowledge it would be safe to consider the value N as a *segregating index* as suggested by WRIGHT. It measures the freely segregating units, instead of all segregating factors. Each unit would be assumed to have at least one locus modifying body size. The computed segregating units, approximately 11, seems not unreasonable in comparison with minimal number of 19 factors in FALCONER'S experiment and 54 factors in MACARTHUR'S experiment as estimated by FALCONER (1953).

One might ask if there is a major locus which modifies body size on either plus or minus direction. In view of the fairly symmetrical distributions of the hybrids, such a possibility would be practically eliminated. The data do not permit a choice between the alternatives, equal effect and effect in geometric series. Still another possibility exists, that is the gene effects are so different and so irregular that they would be difficult to describe mathematically. According to FALCONER's selection experiment the rate of progress in each generation appeared fairly even. This may suggest a possibility of equal gene effects. Mice in the present experiment were genetically so different from FALCONER's that without further experimentation similar interpretation would hardly be justified.

One powerful method for determining the relative effects of genes on body size is to make repeated backcrosses using a marker gene closely linked to one for body size. Because the character of body size is polygenic, relatively small effects could be produced by one or two gene substitutions. The detection of such a marker gene would not be an easy task.

SUMMARY

A study of the genetics of body size in mice by crossing Large and Small strains of mice to produce F_1, F_2 and backcross generations has been made.

An analysis of the means and variances of 60-day body weight in the different genotypic groups of mice has been carried out. Means of F_2 and backcross generations were slightly above their respective theoretical values. The F_1 and F_2 means were halfway between the parents and the backcross means were halfway between the respective parents. The variance of the F_2 hybrids was the largest, the first backcross generations the next and the average of the variances of F_1 hybrids and the Large and Small strains were the smallest.

The effective size genes involved in this cross have been found to act additively on a logarithmic scale rather than arithmetic scale. The computed value of additive genetic variance was .00189 and of the environmental variance within litter was .00041. The overall environmental variance was .00198. The dominance effect contributed to the total variability, if any, was considered to be trivial.

The minimum number of segregating units has been estimated at 11. Each segregating unit has been assumed to include at least one locus modifying body size. The possible interpretation of distribution of genic effect was proposed.

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ESTIMATION OF THE NUMBER OF LETHAL ALLELES IN A PANMITIC POPULATION OF APIS MELLIFERA L.

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BEEKEEPERS have long been familiar with a poor type of brood which due to its scattered appearance is sometimes called "shot brood". An explanation for this character was given by MACKENSEN (1951). MACKENSEN inseminated several queens with semen of their brothers, and by brood viability studies obtained a 1:1 ratio between high (close to 100%) and low (close to 50%) viable brood. He suggested that a series of haploviable homozygous lethals exists so that all females are s_1s_2, s_2s_3, s_3s_4, etc. . . . and the males s_1, s_2, s_3, $\cdots$ s_n. Any female s_1s_2 mated with s_3 males would produce highly viable brood and any s_1s_2 female mated either to s_1 or s_2 males would produce brood of 50% mortality.

MACKENSEN suggested further that the inviable combinations (s_1s_1, s_2s_2, etc.) could be males and, if so, the sex determination mechanism in *Apis mellifera* L. would be the same as the one in Habrobracon except that the alleles are apparently completely lethal when homozygous. This last point of view, that homozygotes may be males, is a question of fact and acceptance of it must await proof that the homozygous dying larvae are male ones. Regardless of whether the series are sex alleles or whether they are only lethal alleles this discovery is outstanding. A queen showing a low degree of brood viability builds such small populations that the colony produces a small amount of honey; and the queen is likely to be superseded, which also lowers the yield.

The studies herewith presented have the scope of estimating the number of these haploviable homozygous lethals in a random mating population of *Apis mellifera* L. so that a better understanding of the population genetics of these bees can be attained.

DEVELOPMENT OF PERTINENT FORMULAE

In undertaking these studies two mathematical problems presented themselves. One was to establish the theoretical frequency of each s allele when the population is in equilibrium, and the other was to find a good estimator for the number of such alleles.

The equilibrium frequencies of the s alleles may be obtained directly from the theory already worked out for selection when the homozygote is at a disadvantage (see WRIGHT 1949, p. 372; LI 1954, p. 260). Suppose the population has n different s alleles, s_1, s_2, $\cdots$ s_n with frequencies x_1, x_2, $\cdots$ x_n ($\Sigma x_i = 1$). If we take the formula for equilibrium given in the above references and assume that each allele has

[1] At present under a Rockefeller Foundation fellowship in the Univ. de Sao Paulo.

[2] Sequence of authors does not imply seniority.

the same selective disadvantage when homozygous, we are led immediately to the formula, $x_i = 1/n$.

This proves that all alleles in a panmitic population, if all of them have the same adaptive value, reach an equilibrium when all alleles have the same frequency.

This is very easy to understand in a practical way: if one *s* allele becomes more frequent in one generation it would have a greater chance of uniting with another like itself thus originating a lethal homozygous combination which would decrease its number in the following generation.

If all *s* alleles have the same frequency, establishing the frequency of one *s* allele would give us the frequencies of all the others. This conclusion determined our method of work which was designed to detect the frequency of any two alleles taken at random from a random mating population.

The second problem was to find an estimator for the number of *s* alleles in the population when each queen is inseminated with semen from a single drone. It is shown in the appendix that a satisfactory estimator is given by

$$n^* = 2\frac{N+1}{H+1}$$

where N is the number of colonies and H is the number of colonies where the *s* allele from the drone is identical with one of the alleles of the queen.

MATERIAL

It is necessary to be careful in choosing a random mating population. The formulae obtained are good only for populations that are random mated for several years, so that they can be said to be in equilibrium. One condition of random mating in honeybees is not to have interference with the raising of new queens.

The bee population studied here belonged to MR. RICARDO GOSSER (Piracicaba, Brazil), a beekeeper who has kept 65 colonies in one apiary for more than 10 years, never rearing queens artificially. The queens mate freely, and within a radius of 10 km (about 6 miles) there are more than 200 other hives. The 65 colonies studied belonged, with 3 exceptions, to the black Portuguese and German races. These 3 exceptions were hybrids in different degrees between black and Italian bees. Practically all 65 colonies showed very good brood with no signs of either 50% or 75% viability. Nine of the 65 colonies showed signs of having superseded the queen shortly before they were used. One of the colonies did not have larvae for grafting in our first collecting of samples because it had a new queen that had not started laying.

METHODS

Queens were reared from as many different colonies of the one apiary as possible, and were inseminated with semen of single drones reared from just one colony. This was accomplished as follows (for details see LAIDLAW 1953):

Four larvae from each colony were "grafted" into artificial queen cups. These grafted cups were put into a "swarm box" for 24 hours and afterwards into a "cell fin-

isher colony" for nine days. On the tenth day the queen cells were transferred to individual screen cages of about 2 × 3 × 5 cm; all cages were put into a frame and placed in a queenless "nursery colony" where the queens emerged and were cared for by the bees until they reached four to ten days old. During this age period each queen was artificially inseminated once with semen of one drone. Before collecting the semen precaution was taken to be certain that the drone had a large amount of semen so that a good insemination could be assured. In the inseminations LAIDLAW equipment and technique (LAIDLAW 1949) were used. After the queens were inseminated the wings were clipped and the queens were put back into the "nursery colony" for one or two days, and were then introduced into queenless nuclei by means of special cages. Two to four days later each queen was released followed by a light

TABLE 1

First and second brood counts and viability percentages. Those with less than 50% are given in parentheses

Hive no.	Number of eggs	Number of worker pupae	Percent of viability	Hive no.	Number of eggs	Number of worker pupae	Percent of viability
D 0-1	507	467	92.11	R 20-2	461	461	100%
R 46	884	396	(44.80)	R 45-1	101	80	79.21
R 68-1	563	408	72.47	R 57-1	550	528	96.00
D 0-2	—	—	100%	R 51-1	438	399	91.10
R 21	1620	1402	86.54	R 24-1	1332	1179	88.51
R 64	2047	944	(46.12)	R 62-1	170	145	85.29
R 40	418	371	88.76	R 9-2	508	475	93.50
R 42	693	572	82.54	R 33-1	186	166	89.25
R 41	1924	945	(49.12)	R 8-2	500	443	88.60
R 59-1	224	167	74.55	R 58-1	1050	1041	99.14
R 7	706	636	90.08	R 56-1	177	150	84.75
R 49-1	178	135	75.84	R 11-2	1671	1431	85.64
R 52	707	543	76.80	R 59-2	600	505	84.17
R 4	—	—	100%	D 2-1	582	563	96.74
R 54-1	1026	973	94.83	R 13-2	1307	602	(46.06)
R 16-1	769	667	86.74	R 28	209	65	(31.10)
R 43	307	147	(47.88)	R 53	371	309	83.29
R 14-1	586	533	90.96	R 35	810	332	(40.99)
R 32	336	316	94.05	R 68	351	279	79.49
R 65	678	643	94.84	R 60	634	520	82.02
R 3	247	180	72.87	R 25-2	643	214	(33.28)
R 55-1	792	691	87.24	R 30	688	632	91.86
R 11-1	489	495	100%	R 15	229	149	65.07
R 9	491	454	92.46	R 34-1	85	76	89.41
R 20-1	559	549	98.21	R 26	278	179	64.39
R 38-1	567	511	90.12	D 0-3	258	187	72.48
R 10	368	306	83.15	R 61	365	154	(42.19)
R 24-2	397	238	59.95	R 6	1056	853	80.78
R 8-1	376	355	94.41	R 29	622	500	80.39
R 48-1	429	412	96.04	R 36	433	343	79.21
R 48-3	296	219	73.99				

spraying of queen and bees with 30% to 50% sugar syrup. All drones used were reared by one colony, colony 39, so they were either s_1 or s_2 as far as their s alleles were concerned. To obtain drones a frame containing sealed drone brood of colony 39 was put into a droneless Italian colony for emergence and the hive entrance was covered with excluder. Even if some indigenous drones occurred in this hive they could be detected by the abdominal color. One to four days after emergence the drones were put into small screen cages, 50 drones per cage, and were left to mature in the same nursery colony as the queens until used. The queens while being inseminated were anesthetised with carbon dioxide. Forty-eight hours after insemination they received an additional CO_2 treatment of 10 minutes duration so they would lay earlier (MACKENSEN 1947). They started laying from 4 to 15 days after insemination.

A few days after a queen started laying an empty comb was given to her. One or two days later the comb was taken out, marked, and the eggs were counted with the aid of a hand counter and the counts were recorded. The comb of eggs was then given to a strong two-story hive in which the queen had been confined by a queen excluder in the lower story. A second count was made when the brood was sealed. In almost all cases of 50% viability the counts were repeated once or twice.

Sixty-one queens were reared from the 65 colonies, ten of which were duplications. Fifty-nine of these were tested as described above. Included in the results are two queens (DO-2 and R4 in table 1) that showed 100% viable brood by inspection but actual counts were not made. Figure 1 illustrates the difference in appearance of 50%

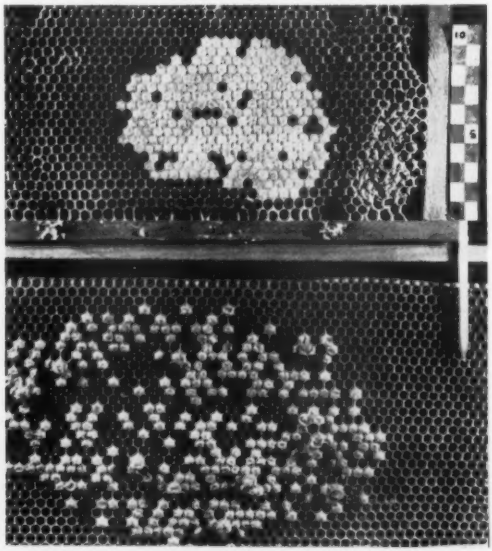

FIGURE 1.—Appearance of 100% viable brood, upper comb, and 50% viable brood, lower comb.

and 100% viability, the upper frame representing 100% viable brood and the bottom one 50% viable brood.

RESULTS AND CONCLUSIONS

The results are summarized in table 1. All percentages of viability smaller than 50% are in parentheses. The figure for R 11-1, where the egg count was smaller than the sealed count, is due to counting mistakes.

A total of 61 queens was analyzed. Nine of these had brood viability ranging from 31.10% to 49.12% and 52 from 59.95% to 100%, table 1.

The bimodal character of the distribution is shown by the graph of figure 2. The only value in the 50% to 60% class is almost 60% and there is little chance that highly viable brood was included with that of low viability.

Applying the formulae developed in the appendix:

$$n^* = \frac{2(N+1)}{H+1},$$

$$V(n^*) = \frac{n^2(n-2)}{2(N+2)},$$

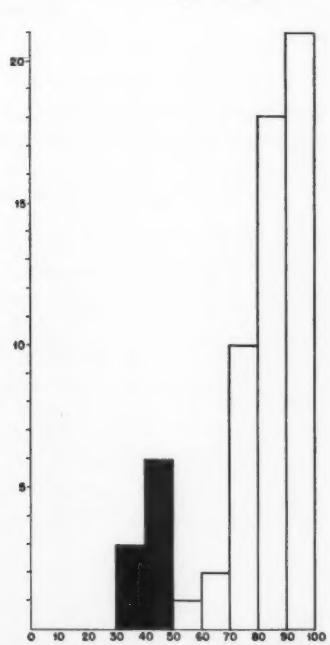

FIGURE 2.—Graph showing the bimodal distribution of different degrees of sterility. The only value in the class 50–60 is 59.9, which is almost in class 60 to 70.

where n = number of different s alleles, N = total number of hives, H = number of hives showing 50% of viability, we have

$$n^* = \frac{2(61 + 1)}{9 + 1} = 12.4,$$

$$s(n^*) = \sqrt{\frac{153.76 \times 10.4}{126}} = \sqrt{12.69} = 3.56.$$

This indicates the number of s alleles in the panmitic population studied is 12.4 with a standard deviation of 3.56.

ROBERTS and MACKENSEN (1951) calculated that with single drone matings and eight s alleles 25% of the colonies should show 50% viable brood; with $n = 16$ this figure dropped to 12.5%. In the case of double mating and 12 s alleles 2.8% of the colonies should show 50% viability and 27.8% should show 75% viability.

These percentages did not occur in the natural population studied but the reason was evident in the experiments. Practically all nuclei showing 50% viability had the queens superseded so quickly that in some cases second counts could not be taken. This contributes to maintain the number of s alleles as large as possible.

Any new allele that appears in the population by mutation has every chance to become established.

SUMMARY

A series of haploviable homozygous lethals was discovered by MACKENSEN (1951) in *A. mellifera* L. and in this paper an estimate of the number of such alleles in a panmitic population is given.

It was demonstrated that if there are n alleles in the population equilibrium is attained when all alleles have the same frequency $\left(\frac{1}{n}\right)$.

An estimator for the number of different s alleles was derived. This was found to be $n^* = \frac{2(N + 1)}{H + 1}$, and the variance to be $V(n^*) = \frac{n^2(n - 2)}{2(N + 2)}$ where N = total number of hives and H = number of hives showing 50% viability.

The material used was 65 hives of a random mating population from which 61 samples (virgin queens) were taken. These queens were inseminated with just one drone each from one colony of the same population. Brood viability tests were made revealing that nine queens had low viable brood and 52 high viable brood. Applying the formulae, 12.4 s alleles were found, with a standard deviation of 3.56. Conditions are offered in a bee population to quickly establish any new s allele arising by mutation.

RESUMO

Este trabalho trata da estimação do número de aleles múltiplos, s, em uma população panmítica de *Apis mellifera* L., do tipo de homozigotos letais descoberto por MACKENSEN (1951).

Demonstra-se no trabalho que quando há n aleles na população, o equilibrio é

atingido quando todos têm a mesma frequência $1/n$. Uma estimativa n^* para o número n de aleles s é dada pela fórmula:

$$n^* = \frac{2(N+1)}{H+1}$$

onde N é o número de colmeias do experimento e H é o número de colmeias em que apenas 50% da prole sobrevive. A variância de n^* é dada pela fórmula

$$V(n^*) = \frac{n^2(n-2)}{2(N+2)}$$

Um experimento foi feito com 65 colmeias de uma população com fecundação ao acaso há muitos anos. Dessa população, 61 raínhas virgens foram tomadas e inseminadas artificialmente, cada uma com semen de um único zangão. Todos os zangões provieram de uma mesma colmeia. Das 61 raínhas, 52 deram prole com alto índice de sobrevivência (perto de 100%) e 9 com baixo índice de sobrevivência (menos de 50%). A aplicação das fórmulas nos deu $n^* = 12{,}4$ aleles com desvio padrão = 3,56.

Sugere-se que qualquer novo alele s aparecido por mutação tem tôda facilidade de se estabelecer na população.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to DR. ERICO AMARAL for his personal help and for the means put at our disposal to accomplish this problem and to MR. RICARDO GOSSER for allowing us to use his hives. Also, without the aid from the Rockefeller Foundation and from the Conselho Nacional de Pesquisas (Brazilian Research Council) this work could not have been done.

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APPENDIX

Statistical aspects of the estimation of the number of alleles

The second problem was to find an estimator for the number of s alleles in the population when each queen is inseminated with semen from just one drone.

While developing the theory, it should be kept in mind that the experiment was carried out with 61 colonies of bees and led to an estimate of about 12 alleles. This knowledge is not essential, of

course, for the derivation of the estimator, but it is important when discussing the degree of approximation of the formulae obtained.

Let us assume that there are n equally frequent s alleles in the population. Then the probability p that the s gene from the drone is identical to one of those from the queen (event A) is $p = \frac{2}{n}$, and the probability of the complementary event (event B) is $q = \frac{n-2}{n}$.

Hence the frequency function $f(x)$ will be $f(x) = p^x q^{1-x}$, $x = 0, 1$, where $x = 1$ denotes event A and $x = 0$ denotes event B. If we now consider N colonies the likelihood will be $L = p^H q^{N-H}$, where $H = \Sigma x$ is the number of colonies where the s allele from the drone is identical with one of the alleles of the queen.

Let us try to apply the method of maximum likelihood to obtain an estimator for n. It is easier to use $\log L$ instead of L itself, so we set up:

$$\log L = H \log p + (N - H) \log q,$$

$$\frac{d}{dn} \log L = \frac{2}{n}\left(-\frac{H}{2} + \frac{N-H}{n-2}\right).$$

By equating this derivative to zero we obtain the estimator

$$\hat{n} = \frac{2N}{H}.$$

Let us now study the properties of this estimator. First of all, we are supposed to calculate its expectation $E(\hat{n})$ to see if it is biased. Now we have a difficulty, for the estimator cannot be used for $H = 0$. But we may take this case, the probability of which is q^N, and consider

$$E(\hat{n}) = \frac{2N}{1 - q^N} \sum_{H=1}^{N} \frac{1}{H} \binom{N}{H} p^H q^{N-H},$$

where $\binom{N}{H}$ denotes the binomial coefficient.

To carry out the summation we can use the identity

$$\frac{1}{H} = \frac{1}{H+1} + \frac{1}{(H+1)(H+2)} + \frac{2}{H(H+1)(H+2)},$$

from which we obtain the approximate formula

(I) $$\frac{1}{H} \approx \frac{1}{H+1} + \frac{1}{(H+1)(H+2)}.$$

$$\frac{1}{1-q^N} \sum_{H=1}^{N} \frac{1}{H+1} \binom{N}{H} p^H q^{N-H} = \frac{1}{(1-q^N)(N+1)p} [1 - q^{N+1} - (N+1)pq^N]$$

$$\approx \frac{1}{(N+1)p} - \frac{q^N}{1-q^N}.$$

Let us put

$$y = \frac{q^N}{1-q^N}$$

and assume that $n < \frac{N}{4}$. This condition can be always fulfilled through the increase of the number N of colonies. Then we have

$$q < 1 - \frac{8}{N} \text{ and } y < \frac{1}{\left(1 - \frac{8}{N}\right)^{-N} - 1} < \frac{1}{e^8 - 1} \approx \frac{1}{2980}.$$

This term is negligible, since for $\frac{N}{6} < n < \frac{N}{4}$ we have

$$\frac{1}{(N+1)p} = \frac{n}{2N+2} > \frac{N}{12N+12} \approx \frac{1}{12}.$$

Hence

$$\frac{1}{1-q^N} \sum_{H=1}^{N} \frac{1}{H+1} \binom{N}{H} p^H q^{N-H} \approx \frac{1}{(N+1)p}.$$

Similarly we obtain

$$\frac{1}{1-q^N} \sum_{H=1}^{N} \frac{1}{(H+1)(H+2)} \binom{N}{H} p^H q^{N-H} \approx \frac{1}{(N+1)(N+2)p^2},$$

therefore

$$E(\hat{n}) \approx 2N \left[\frac{1}{(N+1)p} + \frac{1}{(N+1)(N+2)p^2} \right]$$

$$\approx n \left[1 + \frac{n-2}{2(N+1)} \right]$$

It is evident, therefore, that there is a bias

$$b = \frac{n(n-2)}{2(N+1)}.$$

This bias approaches zero when $N \to \infty$, so that the estimator is asymptotically unbiased. However, for $n = 12$, $N = 60$ the bias is $b \approx 1.0$.

We can try to improve the formula obtained to make it unbiased or, at least, with a smaller bias. We suggest the formula

$$n^* = 2 \frac{N+1}{H+1},$$

which can be used for $H = 0$ and for which we have

$$E(n^*) = n - nq^{N+1}.$$

For $n = 12$, $N = 60$ the bias is now $b = \frac{1}{5634}$, which is negligible.

We shall show now that the estimator n^* is sufficient (KENDALL, vol. II, p. 8; CRAMER, p. 488; Mood, p. 151).

Let $x_1, x_2, \cdots, x_N$ be the observed values of x. We have:

$$L(x_1, x_2, \cdots, x_N \mid n) = p^H q^{N-H},$$

where $H = \Sigma x$ and L denotes the likelihood. Also

$$L_1(n^* \mid n) = \binom{N}{H} p^H q^{N-H}$$

$$L_2(x_1, x_2, \cdots, x_N \mid n^*) = \frac{L}{L_1} = \frac{1}{\binom{N}{H}}.$$

Since $H = \dfrac{n^*}{2(N+1)} - 1$, we see that L_2 does not depend on the values of x, but just on the estimate n^*. Hence it is sufficient. The variance of n^* will be given by the formula

$$V(n^*) = E(n^{*2}) - n^2,$$

for the bias is so small that we may take $E(n^*) = n$. But

$$E(n^{*2}) = 4(N+1)^2 \sum_{H=1}^{N} \frac{1}{(H+1)^2} \binom{N}{H} p^H q^{N-H}.$$

We may use again formula (I) and take

$$\frac{1}{(H+1)^2} \approx \frac{1}{(H+1)(H+2)} + \frac{1}{(H+1)(H+2)(H+3)}.$$

We obtain:

$$E(n^{*2}) = \frac{4(N+1)}{(N+2)p^2} + \frac{4(N+1)}{(N+2)(N+3)p^2}$$

$$\approx n^2 \left[1 + \frac{n-2}{2(N+2)} \right],$$

rom which we conclude that

$$V(n^*) \approx \frac{n^2(n-2)}{2(N+2)}.$$

Note that for $n = 2$ the variance is zero, which should be expected, for then event B is impossible, so that we must have $H = N$, $n^* = 2$.

Let us now study the efficiency of the estimator n^*. It is known (CRAMER, pp. 486–487; KENDALL, pp. 17–20) that for any estimator $\hat{n}$ of n we have

$$V(\hat{n}) \geq \frac{\left(1 + \dfrac{db}{dn}\right)^2}{-N \displaystyle\sum_x \left(\frac{d^2}{dn^2} \log f \right) f}$$

and that equality holds only for efficient estimators. For n^* we have

$$b = -nq^{N+1},$$

$$\frac{db}{dn} = -q^N \left[q + \frac{2}{n}(N+1) \right].$$

For $n = 12$, $N = 60$, the absolute value of this expression is less than 0.0002. Also

$$\frac{d^2}{dn^2} \log f(x) = \frac{1}{n^2} - \frac{1-x}{(n-2)^2},$$

$$-N \sum_{x=0}^{1} \left[\frac{1}{n^2} - \frac{1-x}{(n-2)^2} \right] p^x q^{1-x} = \frac{2N}{n^2(n-2)},$$

so that we have very closely

$$V(\hat{n}) \geq \frac{n^2(n-2)}{2N}.$$

This last expression is practically identical to the one obtained for $V(n^*)$, so that n^* is an efficient estimator.

EVIDENCE FOR GENETIC HOMOLOGY BETWEEN CHROMOSOMES I AND IV IN DROSOPHILA MELANOGASTER, WITH A PROPOSED EXPLANATION FOR THE CROWDING EFFECT IN TRIPLOIDS[1]

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FROM a consideration of chromosome evolution in Drosophila, it has been suggested (MULLER and PAINTER 1932) that in the basal heterochromatin, regions of genetic homology might exist between all of the chromosomes in *D. melanogaster*. In a cytological study of the chromocenter in salivary gland nuclei, PROKOFYEVA (1935) describes connections (which she interprets to be conjugations of homologous loci) between all of the chromosomes in the proximal heterochromatic regions. Other workers have also suggested a meiotic relationship between non-homologous chromosomes (SCHULTZ 1951; LINDSLEY 1955). Genetic evidence for homology specifically between the X chromosome and chromosome IV has been reported by GERSHENSON (1940), LINDSLEY and NOVITSKI (1953), and PARKER (1954).

Further evidence for genetic homology between the X chromosome and chromosome IV is presented below. It will be shown, in addition, that the expectations from triploid females based upon assuming a general homology between all of the chromosomes (the homology between homologous chromosomes being much stronger than that between non-homologous chromosomes) are realized experimentally (BRIDGES and ANDERSON 1926; BEADLE 1935) and that, therefore, general homology between the chromosomes of Drosophila would account for the observed "crowding effect" in triploids.

EVIDENCE FOR X-IV HOMOLOGY

Rather convincing evidence for homology between chromosomes I and IV in the male has been reported by GERSHENSON (1940), who followed simultaneously the segregation of the sex chromosomes and the fourth chromosomes in triplo-IV males which carried, in addition to the normal X and Y chromosomes, a "deleted X chromosome" (essentially an X chromosome heterochromatic fragment). In this study it was found that (for three of four deleted X chromosomes tested) the sex and fourth chromosomes did not segregate randomly, but, rather, the deleted X chromosome tended to go to the pole opposite from that to which two of the three fourth chromosomes went. GERSHENSON tends to rule out the possibility that all three of the deleted X chromosomes were aberrations involving chromosome IV (although the deletions were indeed induced by irradiation), and interprets the results to mean that the deleted X chromosome and chromosome IV are pairing in some proportion of the meioses and then disjoining.

The following experiment was performed for the purpose of testing for X-IV

[1] This investigation was supported by a research grant C-1578(3C) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

TABLE 1

The results from crosses of (A) diplo-IV and (B) triplo-IV females carrying normal X chromosomes to y B males carrying the attached XY (= YSX·YL) chromosome and a normal Y chromosome

Constitution of parental female	Number of progeny			
	Regular		Exceptional	
	B ♀♀	+ ♂♂	+ ♀♀	*y B* ♂♂
(A) +/+; sv^n/sv^n	1,138	1,157	2	3
(B) +/+; $sv^n/sv^n/sv^n$	2,196	2,476	18	14

A two by two contingency test on the difference between the two experiments gives a χ^2 value of 6.3 with 1 degree of freedom, and hence $.02 > P > .01$.

homology in the female, and simultaneously in a situation which involved only normal chromosomes. The experimental setup was as follows. Triplo- and diplo-IV sibling females carrying normal, wild type, X chromosomes were crossed to YSX·YL, *y B* males which also carried a normal Y chromosome. The results from these matings are given in table 1. The rationale behind this experiment was simply that if there exists some homology between the X chromosome and chromosome IV, then, in the triplo-IV females, the extra fourth chromosome might occasionally pair with one of the X chromosomes and thereby cause nondisjunction of the X's, an occurrence which is detectable in this experiment. It can be seen from table 1 that there are, in fact, significantly more exceptional offspring in the triplo-IV run than in the control diplo-IV set.

One further point ought to be noted about this experiment. The diplo-IV from triplo-IV classification (for the purpose of obtaining the parental females) was made possible by the use of the fourth chromosome marker sv^n which appears more wild type in triplo-IV flies than in diplo-IV flies. It was found, however, (after the experiment just described was completed) that this classification is by no means perfect in the stocks in this laboratory, and some errors, therefore, may very possibly have been made. However, an error in either direction (i.e. a diplo-IV which was classified as a triplo-IV or *vice versa*) would tend to obscure the difference between the experimental and control sets. This is so for the reason that triplo-IV females which were put in the diplo-IV set would increase the nondisjunction in that set, whereas diplo-IV females placed in the triplo-IV run would tend to decrease the amount of nondisjunction in that experiment. Thus it would appear that the difference between the two sets which has been observed is a minimum difference, with the true difference very likely being larger.

One other type of experiment is rather suggestive in this connection. Although the frequency of X-ray induced translocations in female Drosophila is known to be extremely low, reasonably large numbers of induced X-IV exchanges have been reported between the YSX·YL chromosome and chromosome IV (LINDSLEY and NOVITSKI 1953) and between attached-X chromosomes and chromosome IV (PARKER 1954), these exchanges being induced in females. That this might be indicative of X-IV ho nology has been suggested in both of these works.

HERSKOWITZ (1954), and ABRAHAM, HERSKOWITZ and MULLER (1954) irradiated

females carrying an attached-X chromosome with no Y chromosome. They report that, of 39 attached-X detachments analyzed, 16 involved rearrangements with chromosome IV, and suggest that this may be due to "proximity of breakages" in these chromosomes. Such proximity could result from homology between chromosomes I and IV.

DISCUSSION

From the information presented above, it is reasonably clear that regions of homology are shared between the sex chromosomes and chromosome IV. There are a number of general arguments which suggest that (a) these regions of shared homology are located in the basal heterochromatin, and (b) this homology is shared by all of the chromosomes in *D. melanogaster*, and is not restricted only to chromosomes I and IV. One such argument has been presented in detail by MULLER and PAINTER (1932) and hence need not be considered again here. Another argument for general homology is simply that the mere presence of a chromocenter in Drosophila might be said to imply the existence of shared regions of homology in the basal heterochromatin. This implication becomes, of course, very much stronger in the light of PROKOFYEVA'S (1935) description of conjugation among all of the chromosomes in the chromocenter. Finally, a rather fascinating argument has been presented by GERSHENSON (1940), who suggested that the lack of detected genetic markers in the heterochromatin might come about as the result of polyploidy for genes in these regions. That is, that there are representatives of the same loci in the heterochromatin of all of the chromosomes thereby causing (a) homology between these segments, and (b) a high degree of polyploidy for these loci, thus rendering their detection very improbable by ordinary genetic methods.

The most obvious place to look for evidence for general homology is, however, in the meiosis of triploid females. In such females there ought to be a great many univalent heterochromatic regions available for pairing with the heterochromatin of non-homologous chromosomes. A consideration of the expectations from triploids, assuming that this were the case, follows.

In a triploid there are present three homologs of each chromosome of the set. If the separation at the first meiotic division involves two homologs from one, with each set of three behaving independently of every other set of three, then the frequencies of haploid gametes, diploid gametes, and, in fact, any specific combination of chromosomes is easily predicted algebraically. These frequencies are all $(1/2)^n$ where n is the haploid number. In Drosophila, it has been known for a long time that the distribution to the gametes of chromosomes in triploids differs markedly from such randomness (BRIDGES and ANDERSON 1926; BEADLE 1934, 1935). This departure from randomness may be considered as a tendency for equal numbers of chromosomes to proceed to the poles during meiosis (the "crowding effect"). Since, however, only gametes with balanced complements can produce viable offspring, the observation essentially is that 2X:1A gametes are produced more frequently than 2X:2A gametes, whereas 1X:2A gametes are more frequent than 1X:1A gametes. This has been shown to hold for the case of free X triploids by BRIDGES and ANDERSON (1926), and to a lesser, but nonetheless quite significant, degree for the case of attached-X tri-

ploids by BEADLE (1934, 1935). For free X triploids, BRIDGES and ANDERSON (1926) found that the frequency of 2X:1A gametes, recovered as diploid females after fertilization by Y-bearing sperm, was 1,116 as compared with 154 recovered diploid females resulting from the fertilization of 1X:1A gametes by X-bearing sperm. The same comparison for the case of attached-X triploids (BEADLE 1935) was 1,968 2X:1A to 1,186 1X:1A. The other possible comparison, that is 2X:2A as compared with 1X:2A gametes, is made in intersexes, and is 104 to 898 respectively for free X triploids, and 700 to 1,854 for attached-X triploids.

Thus it can be seen that if an egg receives two sets of major autosomes, then it is much more likely to receive but a single X chromosome, whereas if it receives only one set of autosomes, then it is most probable that it will receive two X's.

These inequalities have been considered by BEADLE to be a consequence of the size and mass of the chromosomes causing an orientation on the metaphase plate. A somewhat simpler explanation for this crowding effect is available, since it can be seen that this sort of discrepancy is that which would be expected if pairing in a triploid is considered to be a situation in which each set of homologs forms a bivalent plus a univalent. If there were a general homology between all of the chromosomes, the univalents would be expected to pair with, and separate from, each other. Since if univalent chromosomes II and III separate from each other no viable offspring can result, the overall observed results would be those outlined above. It is to be noted that the bivalent-univalent situation postulated here refers only to the heterochromatic regions.

The difference in the degree of the discrepancy between free X, as compared with attached-X, triploids can be rationalized by assuming that in the attached-X case the compound and the single X often form what is essentially a simple bivalent, thus reducing the amount of pairing between the X chromosome and chromosomes II and III, which would indeed reduce the extent of the discrepancy.

One other result from triploids tends to confirm the above arguments. It has been shown by BEADLE that the frequency of crossing over in attached-X triploids, between the free X and the attached X's, in the vicinity of the centromere (proximal to *f*), is about half as great when the observations are confined to the 1X:2A and 2X:1A gametes than they are when observed in 1X:1A and 2X:2A gametes. This is an expected result if, during the meiotic divisions, there is a tendency for the attached-X to pair with, and disjoin from, a major autosome, since such pairing would interfere with pairing between the free X and the attached-X. Under these conditions the attached-X would disjoin both from the free X and the major autosome, and hence a depressed frequency of crossing over would be observed in precisely the gamete types 1X:2A and 2X:1A In fact, the depression is 50 percent (14 versus 7–8 percent) suggesting that when the attached-X and an autosome are paired, only one of the two arms of the attached-X is available for exchange with the free X.

SUMMARY

1. The evidence for homology between chromosomes I and IV is summarized. It is shown that primary nondisjunction is greater in triplo-IV females than in normal

diplo-IV's. This is interpreted to mean that the extra fourth chromosome occasionally pairs with, and hence causes nondisjunction of, the X chromosomes.

2. It is shown that previously reported results from triploid females agree with the expectations based upon assuming general homology among all of the chromosomes in Drosophila, if (a) homologous pairing is stronger than non-homologous association, and (b) the regions of shared homology are located in the heterochromatic regions; these regions being responsible for the patterns of disjunction. It is suggested that such general homology is sufficient to account for the crowding effect phenomenon manifest in triploids. It is also shown that general homology can explain the differences in crossover values observed in the various gamete types produced by triploid females.

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FURTHER NOTES ON THE NATURE OF NON-RANDOM DISJUNCTION IN DROSOPHILA MELANOGASTER[1]

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THE nature of meiosis in ordinary diploid organisms is such that homologous chromosomes are usually present in gametes with equal frequencies. One exception to this rule in *Drosophila melanogaster* has been termed non-random disjunction (NOVITSKI 1951). Here it has been found that homologs are recovered unequally frequently from females when they are heteromorphic, or of unequal size. When this is the case, the smaller of the two homologs is recovered about twice as frequently as the larger. The genetic analysis has suggested, as the basis for this phenomenon, the formation of an asymmetric dyad as a consequence of crossing over, followed by the preferential inclusion into the egg nucleus of the smaller of the two chromatids making up the dyad. It was suggested, at the time the phenomenon was first described, that one might imagine this result to be brought about by the dragging of the longer chromatid of the dyad at first anaphase, leading to an orientation of the centromere region at second metaphase of such a nature that the shorter chromatid would tend to become included in an egg nucleus. Such a sequence of events is purely hypothetical and was proposed primarily as an aid in understanding the genetic results.

The following two points will be considered below. 1. A hypothesis to explain non-random disjunction in mechanical terms is discussed and discarded. The experimental results, however, reveal a deficiency of newly generated single ring X chromosomes from females carrying tandem metacentric compound X chromosomes (for a description of the compound chromosomes, see NOVITSKI 1954) with no homolog. It is shown that this same phenomenon extends to the single rings generated by tandem compound ring X chromosomes, and also to the single rod chromosomes generated by tandem acrocentric compound X chromosomes. 2. Non-random disjunction in the tandem acrocentric compound X chromosome is now demonstrated to occur; its extent being considerably greater than in any previously analyzed case.

THE BEHAVIOR OF TANDEM METACENTRIC COMPOUND X CHROMOSOME WITHOUT AN HOMOLOGOUS CHROMOSOME

A hypothesis to explain, in mechanical terms, the phenomenon of non-random disjunction was tested during the course of the present work. Although the tests did not confirm the initial hypothesis, the results obtained proved to be of considerable interest in another connection and hence are reported here.

The hypothesis which was tested depended upon assuming that paired heterochromatic regions tend to separate later, or with more difficulty, than paired euchro-

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matic regions. If such were the case, and if two homologous chromosomes differed from one another in that one of them had a distally placed heterochromatic segment, then, following a single exchange, the dyads separating from one another are each composed of a longer and a shorter chromatid, the longer, in each case, possessing the heterochromatic distal region. If these heterochromatic regions remain paired for some time after the more proximal regions have separated, then both of the dyads should become so oriented that the shorter chromatid faces an outer, and hence a potentially functional, egg nucleus. This would result in an excess recovery of shorter chromatids, which is the observed result to which the name non-random disjunction has been given. In fact, the structural situation just described (actually sc^4 sc^8/sc^8) represents one of the major evidences for the phenomenon.

An experiment which would test such a hypothesis immediately suggests itself. Asymmetric dyads are produced not only from homologs which differ structurally from one another, but are also generated by some of the compound X chromosomes. One such compound is the tandem metacentric compound X chromosome (known, in older usage, as the tandem attached-X) which produces, following certain types of exchanges, dyads composed of a tandem metacentric compound X chromosome and a single ring X chromosome. These single rings have been shown to disjoin non-randomly from the compound chromosome (NOVITSKI 1951). The tandem metacentric compound used in those experiments was heterozygous for $In(1)sc^8$, and hence possessed a distal heterochromatic segment. The parental females carried, in addition to the compound, a heterochromatic homolog (the Y chromosome) which regularly separates from the compound at anaphase I. A delay in the separation of the Y chromosome from the distal heterochromatin of the compound chromosome would, according to the hypothesis, orient the dyad so as to give the observed excess of recovered single ring X chromosomes. A test of the hypothesis, consequently, is simply to remove the homolog, which should eliminate the orientation, and hence the excess of recovered single rings.

Two points must be mentioned at this juncture. First, an excess in the recovery of single rings is manifest in tandem metacentric data of STURTEVANT and BEADLE (1936). These workers used a tandem metacentric compound which did not possess a distal heterochromatic segment. (Their compound was an attached-X heterozygous for $In(1)y^4$, an inversion which has its distal break to the left of *y* and its proximal break just to the right of *fu*). Since the hypothesis which was tested requires the separation of basal heterochromatin, distally placed on the compound, from the Y chromosome (which was present in the experiments of STURTEVANT and BEADLE) non-random disjunction in this case represents critical negative evidence against the hypothesis. For this reason, a tandem metacentric compound similar to that of STURTEVANT and BEADLE has been constructed and tested. The results obtained are in full agreement with those of the earlier work (see below), and hence the hypothesis would, on this basis alone, have to be discarded. Secondly, it should be noted that after the experiments to be described were completed, further (and extremely convincing) evidence against the hypothesis was reported by WELTMAN (1954) who demonstrated non-random disjunction in an especially constructed attached-X chromosome, tested in females which did not carry a homolog for the attached-X.

In conclusion then, the data of STURTEVANT and BEADLE, the repetition of their results in the present work, and finally the data of WELTMAN are sufficient to eliminate the initial hypothesis. The experiments to be described below therefore, although originally done with this particular hypothesis in mind, are not considered from that point of view.

Experimental results

Two tandem metacentric compound X chromosomes have been used in the present study. The first of these is designated as the *Hw f* tandem metacentric; its origin has been described in detail previously (NOVITSKI and LINDSLEY 1950). The particular properties of this compound which are of interest here are: (1) the single ring X chromosomes produced by crossing over are viable in both males (XY or XO) and females; and (2) the distal portion of one arm of the compound, having been derived from $In(1)sc^8$, possesses a distal heterochromatic segment containing the *bb* locus and the nucleolus organizing region (*NO*).

The second tandem metacentric compound studied, designated here as the y^4 tandem metacentric, had been synthesized in a triploid female carrying an attached-X chromosome homozygous for *y v f*, and a rod X chromosome containing the y^4 inversion. An exchange, in such a female, between the attached-X chromosome and the inverted chromosome, taking place proximal to the y^4 inversion, produced the tandem metacentric compound. The properties of this compound which are of interest here are: (1) the single rings generated by crossing over are deficient for the tip of the X chromosome and duplicated for the proximal uninverted section of $In(1)y^4$, and hence are almost invariably male lethal although they are recoverable in heterozygous females; (2) the compound possesses no distally placed heterochromatin; and (3) the compounds are heterozygous for the markers *v* and *f*.

Data have been collected from three pairs of parallel crosses. First, females carrying the *Hw f* tandem metacentric compound both with and without a Y chromosome were crossed to Canton-S (wild type) males. The results from these matings are given in table 1. A similar pair of matings was made between females carrying the y^4 tandem metacentric compound, both with and without a Y chromosome, by Canton-S males. The results from this pair of crosses are given in table 2. The y^4 tandem metacentric was also tested in females which either carried no homolog for the compound, or which carried FR2 as a homolog. (FR2 is a heterochromatic chromosome fragment equiva-

TABLE 1

The results from crosses of females carrying the Hw f *tandem metacentric compound X chromosome, both with and without a Y chromosome, by Canton-S (wild type) males*

Type of progeny	Phenotype of progeny	Number of progeny from	
		♀♀ with a Y	♀♀ without a Y
Compound X ♀♀	Hw f ♀♀	285	369
Patroclinous ♂♂	+ ♂♂	2,121	2,061
Ring-bearing ♀♀	+ ♀♀	802	570
Ring-bearing ♂♂	f ♂♂	765	598

TABLE 2

The results from crosses of females carrying the y^4 tandem metacentric compound X chromosome heterozygous for v *and* f, *both with and without a Y chromosome, by Canton-S (wild type) males. Occasional crossovers which occurred outside of the y^4 inversion are included with the* y *female class. The numbers in parenthesis are the observed numbers corrected for viability according to the method shown in the text*

Type of progeny	Phenotype of progeny	Number of progeny from	
		♀♀ with a Y	♀♀ without a Y
Compound X ♀♀	y ♀♀	911	764
Patroclinous ♂♂	+ ♂♂	4,734	3,964
Ring-bearing ♀♀	+ ♀♀	1,191 (1,575)	740 (979)
Homozygous ♀♀	y v ♀♀	29	20
	y f ♀♀	0	2

TABLE 3

The results from crosses of females carrying the y^4 tandem metacentric compound X chromosome heterozygous for v *and* f, *both with and without FR2 (a heterochromatic chromosome fragment equivalent to YL and carrying y^+), by* y B *males carrying the YSX·YL chromosome plus a normal Y chromosome. Occasional crossovers which occurred outside of the y^4 inversion are included with the* y *female class. The "patroclinous males" are y^+ when they receive FR2 from the parental females. The numbers in parenthesis are the observed numbers corrected for viability according to the method shown in text*

Type of progeny	Phenotype of progeny	Number of progeny from	
		♀♀ with FR2	♀♀ without FR2
Compound X ♀♀	y ♀♀	923	1,134
Patroclinous ♂♂	(y or y^+) B ♂♂	4,241	5,585
Ring-bearing ♀♀	y B ♀♀	1,249 (1,652)	1,076 (1,423)
Homozygous ♀♀	y v ♀♀	43	45
	y f ♀♀	1	1
Exceptional ♀♀	+ or B ♀♀	10	—
Exceptional ♂♂	y B ♂♂	100	—

lent to the long arm of the Y chromosome and carrying the normal allele of *y* from the distal uninverted section of *In(1)sc*^{8}. These females were crossed to *y B* males carrying a YSX.YL chromosome plus a normal Y chromosome. The results from this pair of experiments are given in table 3.

Although the single rings generated by the y^4 inversion are recoverable in the heterozygous condition as females in spite of the fact that the rings carry a rather sizable duplication, it might be suspected that there would be an adverse effect on the viability of such ring-bearing females. To test for such a possibility (both in the case of the rings from the y^4 tandem metacentric as well as for the case of the *Hw f* tandem metacentric), rings from each type of tandem metacentric were tested in the following way: ring/y^2w^a *B*, M-5 females were crossed to Canton-S (wild type) males. The results from these crosses are given in table 4. The two types of female progeny from this cross (i.e. those which get the M-5 chromosome from the parental female

TABLE 4

Tests for the viability of single ring X chromosomes generated by the Hw f *and the* y^4 *tandem metacentric compound X chromosomes. The cross in both cases was as follows: ring/*y^2 w^a *B, M-5* ♀♀ × *Canton-S (wild type)* ♂♂

Type of progeny	Phenotype of progeny	Ring from *Hw f* tandem metacentric	Ring from y^4 tandem metacentric
Ring-rod ♀♀	+ ♀♀	196	570
Rod-rod ♀♀	B/+ ♀♀	223	754
Ring-bearing ♂♂	f ♂♂	208	0
Rod-bearing ♂♂	y^2 w^a B♂♂	146	522
Exceptional ♂♂	+ ♂♂	0	21

and hence are *B*, versus those which get the ring and are therefore wild type) give a measure of the viability of the ring in each case. In the case of the ring from the *Hw f* tandem metacentric, it can be seen that 196 rings are recovered to 223 rods. Thus the viability of ring-bearing females carrying the ring from the *Hw f* tandem metacentric is not greatly depressed. The ring from the y^4 tandem metacentric, on the other hand, does show a depression. As can be seen from table 4, there were 754 rods recovered as compared with only 570 rings. As a consequence, in analyzing each test involving the y^4 tandem metacentric, the ring-bearing female class must be increased to account for this viability depression. Such a viability correction may be calculated simply according to the relationship: 570:754 = observed number of rings:corrected number of rings. The corrected numbers for the ring classes are given in parenthesis next to the observed figures in tables 2 and 3. It is the corrected figures which will be used in all of the calculations to follow; it should be noted, however, that the conclusions to be drawn from these experiments do not depend upon these corrections.

Analysis of the data

An examination of the data given in tables 1, 2, and 3 reveals a consistent difference between the experiments in which the parental females carried a homolog for the tandem metacentric compound as compared with those in which they did not. Thus for each set of experiments there are proportionately fewer rings produced by females which carried no homolog for the compound than from females which did carry such a homolog.

As a first approach, it might be imagined that this reduction in the recovery of single rings is a reflection of a change in the amount of non-random disjunction; specifically, a reduction or an elimination of such non-randomness (since when non-randomness operates it causes an excess in the recovery of single rings). That this is, in fact, not the case may be shown in two ways, the second of which is of particular interest. First, an examination of the raw data in tables 1, 2, and 3 shows that in every case (of specific interest at present, of course, are those cases in which the parental females carried no homolog for the compound) the number of rings recovered in either sex exceeds the number of compound-X females recovered. This, barring a gross viability depression of the compound-X female class, is equivalent to a direct observation of non-randomness. This follows from a simple consideration of the expecta-

TABLE 5

The ratio of single ring X chromosomes recovered as females to recovered patroclinous males, and the ratio of recovered tandem metacentric compound X chromosomes to recovered patroclinous males for all of the tandem metacentric tests reported. The figure used for the rings generated by the y^4 *tandem metacentric has been corrected for viability by the method shown in text. The column headed "Table" gives the source of the data from which the ratios have been obtained*

Cross	Table	Ring ♀♀ /Pat. ♂♂	Tandem metacentric ♀♀ /Pat. ♂♂
Hw f/Y × Canton-S	1	0.38	0.13
Hw f/0 × Canton-S	1	0.28	0.18
y^4/Y × Canton-S	2	0.33	0.20
y^4/0 × Canton-S	2	0.25	0.20
y^4/FR2 × YSX·YL	3	0.38	0.22
y^4/0 × YSX·YL	3	0.25	0.21

tions from the various types of exchanges (for diagrams, see NOVITSKI 1951): in the absence of non-randomness, from no-exchange tetrads there are produced only compound X's, whereas from the single and double-exchange tetrads there is produced a compound X for each single ring, and, consequently, if there were no non-randomness, there should be at least as many compounds recovered as rings (in either sex). Since an excess of rings is apparent in the data, it may be concluded that non-randomness is operative in tandem metacentric compounds even in the absence of a homolog.

Another way of considering the depression of rings in cases in which the compound-bearing females carried no homolog is as follows. If the depression of rings were a consequence of a shift (of any sort) in the relative recovery of rings and compounds, then, if the relative proportion of rings is reduced, the proportion of compound-X's would go up. In table 5, the ratio of rings to patroclinous males and the ratio of compound X's to patroclinous males is given for all of the cases reported. From this table it can be seen that, in general, the relative proportion of recovered compounds remains constant (considering pairs of parallel runs with and without a homolog), but in every case the proportion of rings is sharply reduced when the homolog is absent. From the data it appears, then, that there is a reduction in the number of rings produced by females carrying a tandem metacentric compound with no homolog, without, however, a concomitant increase in any of the other classes. Since there are more rings than compound-X's observed, and since none of the classes is increased to account for the depression in rings, it would appear most reasonable to attribute this depression to a lethality of some percentage of the rings newly generated by the compound chromosome.

In this connection there is an observation of considerable interest. Crosses have been made of females carrying tandem compound ring X chromosomes both with and without FR2 (NOVITSKI 1954) to identical males. In the case in which the females carried FR2, the ratio of ring males to patroclinous males was 0.33, while the ratio of compound-bearing females to patroclinous males was 0.09. In a parallel run in which the females carried no homolog, the ratios were 0.25 and 0.09, respectively. Hence it appears that in the tandem ring, as well as in the tandem metacentric, there is a depression of recovered single rings when the parental females lack a homolog for the

compound. This observation renders it all the more likely that this depression of rings is a lethality, since the expectations from the various types of exchanges are rather different for the two compounds.

Measurements of the degree of non-randomness

The extent of non-random disjunction is given by the numerical value of the coefficient c, where a value of 0.5 represents random disjunction (no non-randomness) and one of 1.0 represents 100 percent recovery of one of the two chromatids of the asymmetric dyad. In estimating c values in experiments with compounds, it is convenient to derive special equations in order to circumvent difficulties that arise because of the complexity of the genetic set-up. The equation for c for the case of the tandem metacentric had been given earlier (NOVITSKI 1951) as

$$c = \frac{X^c}{2 \text{ Pat. } ♂♂ - XX - X^c} - \frac{E_2(1 - c)}{4E_1 + 3E_2}.$$

The last term in the expression is ordinarily disregarded in calculating, being a correction factor of small magnitude.

However, since the equation was first derived, evidence has been presented indicating that one of the two classes of four strand doubles from the tandem metacentric gives rise to a double second anaphase bridge which, unlike the lethal single second anaphase bridges, yields a nullo-X egg (NOVITSKI 1955). In the absence of a special genetic situation which will distinguish between the patroclinous males that arise regularly in crosses involving females carrying compound X chromosomes, and those from four strand doubles, the observed patroclinous male class will include both types. It is of some importance, therefore, to correct the formulae presented earlier for the tandem metacentric, taking into account the additional contribution to the patroclinous male class from four strand doubles. The equation given previously as

$$\text{Pat. } ♂♂ = \tfrac{1}{2}(E_0 + E_1 + E_2)$$

should be modified to

$$\text{Pat. } ♂♂ = \tfrac{1}{2}(E_0 + E_1 + E_2 + \tfrac{1}{8} E_2).$$

When the final formula for c for the tandem metacentric is modified to take into account this change, it becomes

$$c = \frac{X^c}{2 \text{ Pat. } ♂♂ - XX - X^c} - \frac{E_2(2 - 3c)}{8E_1 + 7E_2}$$

The only difference between this equation and that presented above is in the last term of the expression, the correction factor. Since the absolute magnitude of the revised term must be less than that of the original one, and since this term represents a correction factor of small magnitude indicating an overestimate in the calculated c values, it follows that the equation used earlier to obtain c values for the tandem metacentric is probably more accurate than was thought at the time. Consequently, in the calculations of c values given in table 6, the correction factor has been ignored.

The values for the tests in which the parental females carry no homolog are not

TABLE 6

The c *values for all of the tandem metacentric tests made. The column headed "Table" refers to the table from which the data for the calculations were taken. The formula for* c *is given in text. Rings = 2(ring-bearing ♀♀). X-Y = YSX·YL,* y B

Cross	Table	2(Pat. ♂♂)	2 (compound ♀♀)	Rings	c
Hw f/Y × Canton-S	1	4,242	570	1,604	0.78
y^4/Y × Canton-S	2	9,468	1,880	3,150	0.71
y^4/FR2 × X-Y/Y	3	8,682	1,936	3,322	0.97

given since the reduction of the single ring classes would render the determination of *c* spurious.

One of the *c* values given (that of the y^4 tandem metacentric/FR2 × YSX·YL/Y; table 3) is obviously too high and requires some comment. The patroclinous males from that cross carried the YSX·YL chromosome plus FR2, and might possibly suffer from a viability depression since they would carry a duplication for the *y* region (and possibly a triplication, since the YSX·YL chromosome may itself carry a duplication for the *y* region). If this class were depressed, then the *c* value would indeed be inordinately high. That this is, in fact, very possibly the case is shown by the following argument. If it is assumed that the *c* value for the y^4 tandem metacentric is the same for the case with FR2 (table 3) as it is for the case with a normal Y (table 2) and is equal to 0.71, then it is possible, using the formula for *c*, to get an estimate of the number of patroclinous males that should have been observed in the case with FR2. The observed number of patroclinous males was 4,241, whereas the figure obtained in the manner just outlined is 4,869. The validity of this number can be checked by comparing the ring to patroclinous male and compound-X to patroclinous male ratios. They become 0.33 and 0.19 respectively, agreeing very closely with the other tests recorded. It can be seen that the other *c* values obtained agree very well with previously published values (NOVITSKI 1951).

NON-RANDOMNESS IN THE TANDEM ACROCENTRIC COMPOUND X CHROMOSOME

The tandem acrocentric compound X chromosome regularly generates single X chromatids by crossing over within itself. Asymmetric dyads are thus produced, and it would be expected that the short single X chromatids would be recovered preferentially. However, the earliest results (NOVITSKI 1954) from this compound were interpreted as indicating no non-randomness. It was postulated at that time that the presence of the long arm of the Y chromosome, which was appended as a second arm of the compound, might have been responsible for diminishing the amount of non-randomness, possibly by some effect such as increasing the mass of the dyad and therefore decreasing, percentage-wise, the difference between the two chromatids.

An experiment was therefore set up to determine whether the degree of non-randomness might be affected by the presence of the long arm of the Y chromosome (= YL) carried basally on chromosomes which ordinarily (i.e. when the Y chromosome arm is not involved) show non-randomness. The two X chromosomes chosen differed in that one of them carried FR1 distally. The so-called FR1 chromosome

has been shown to be an X chromosome in normal sequence with the short arm of the Y chromosome at the free end (BRAVER 1955). Females carrying this chromosome, along with the normal one, produce eggs about two thirds of which carry the normal tip and one third, the FR1 tip. The long arm of the Y chromosome was attached to the base of the chromosome carrying FR1 by crossing over, and females carrying this chromosome, and a normal one, were tested. This, then, provides an ideal genetic set-up for determining whether dyads with the long arm of the Y attached basally behave in the same way as those with the normal subterminal X centromere, since both types of dyads are to be found in meioses of a given female. This is the equivalent of both an experimental and control situation in a single individual. The exact genetic constitution of the mating along with the results is given in table 7.

The data have been analyzed in the following way. Equations representing the expected frequencies of noncrossovers, singles, and doubles, under conditions of non-randomness, have been presented earlier (NOVITSKI 1951). These equations have been modified by assuming that there is not just one coefficient of non-randomness, but two; one when the dyad has the normal X centromere, and a second when the dyad carries YL at the base. These two coefficients of non-randomness are labelled c and c', respectively. The unknowns in the equations are c, c', and the frequencies of tetrads with no, one, and two exchanges. The numbers used in the analysis are those given under the male columns, corresponding to the recovered noncrossovers, singles, and

TABLE 7

Progeny of females of the constitution FR1 y cv v f·*YL*/car *mated to* y v car *males. Crossover regions are: 1′*, y-cv; *1″*, cv-v; *1*, y-v; *2′*, v-f; *2″*, f-car; *2*, v-car. *A dash indicates an unidentifiable class, and a vertical arrow through classes points to the more general class for which identification is unambiguous*

Crossover region	FR1 ♂♂	Non-y ♂♂	FR1 ♀♀	Non-y ♀♀
0	859	1,622	959	1,887
1′	201	308	↓	↓
1″	350	478	↓	↓
1	551	786	605	853
2′	328	531	↓	↓
2″	97	120	↓	↓
2	425	651	433	735
1′, 1″	7	11	—	—
1′, 2′	35	69	↓	↓
1′, 2″	22	27	↓	↓
1″, 2′	46	78	↓	↓
1″, 2″	21	35	↓	↓
1, 2	122	209	158	264
2′, 2″	2	8	—	—
Total in ♂♂				
non co	859	1,622		
singles	976	1,437		
doubles	124	217		

doubles, at the bottom of table 7. The maximum likelihood solution of these equations gives the following values: $c=0.652$, $c'=0.650$, $E_0=334$, $E_1=3{,}540$, and $E_2=1{,}350$. The exchange frequencies arrived at in this way represent percentages 6.4, 67.8, and 25.8, respectively. For a somewhat similar crossover experiment performed earlier (NOVITSKI 1951) in which the calculations of exchange frequencies were made by direct algebraic solution (which was not possible in this case) the values were 4.4, 68.4, and 27.1, respectively. This agreement serves to validate the method used. Of greatest interest, of course, is the astonishingly close agreement in the values of non-randomness for the dyads with and without YL. There seems to be no question but that the addition of YL to one of the chromosomes has not changed non-randomness appreciably.

These results indicate that the failure to find evidence for non-randomness in the tandem acrocentric compound (NOVITSKI 1954) was not due to the presence of YL, which was appended as a second arm to the centromere of the compound. This necessitates a re-evaluation of the tandem acrocentric data. The analysis referred to above was based upon an experiment in which approximately 80 percent of the progeny came from compound-bearing females which did not carry a free Y chromosome. As was shown above for the tandem metacentric and the tandem ring, the presence or absence of a Y chromosome in a compound-bearing female may affect the number of single chromosomes produced by crossing over which are recovered. It consequently becomes of some interest to consider results from tandem acrocentric females with and without a Y chromosome. Therefore, a number of tests of tandem acrocentric females, with and without a free Y chromosome, have been made. The results from these tests are given in tables 8 and 9. In table 10 are recorded the c values for these tests. The formula for c for the case of the tandem acrocentric has been given by NOVITSKI (1954). It should be noted, however, that the equation was given incorrectly in that work (although the c values recorded were calculated from the correct formula), and should be

$$c = \text{single chromosomes}/(4 \times \text{Pat. } ♂♂ - 4 \times \text{Tandem acrocentrics} - \text{single chromosomes}) + \text{C.F.}$$

It is this equation (ignoring, as above, the correction factor) which is used for determining the c values given in table 10.

TABLE 8

The results from a mating of females which carry a tandem acrocentric compound X chromosome homozygous for the mutant allele of y, *both with (A) and without (B) a Y chromosome—by* y B *males which carry the YSX·YL chromosome and no homolog*

Type of progeny	Phentotype of progeny	Number of progeny from	
		(A) ♀♀ with a Y	(B) ♀♀ without a Y
Compound X ♀♀	y ♀♀	272	208
Patroclinous ♂♂	y B ♂♂	907	687
Rod-bearing ♀♀	y B ♀♀	614	395
Rod-bearing ♂♂	y ♂♂	600	457

TABLE 9

The results from a mating of females which carry a tandem acrocentric compound X chromosome homozygous for the mutant allele of y *with no homolog, by males carrying the YSX·YL,* y B *chromosome both with (C) and without (D) FR2 (for description see text)*

Type of progeny	Phenotype of progeny	Number of progeny from	
		(C) ♂♂ with FR2	(D) ♂♂ without FR2
Compound X ♀♀	y or + ♀♀	580	561
Patroclinous ♂♂	y B ♂♂	2,113	1,956
Rod-bearing ♀♀	y B ♀♀	902	843
Rod-bearing ♂♂	y or + ♂♂	853	871
Exceptional ♂♂	y ♂♂	3	—
	B ♂♂	2	—

TABLE 10

The c *values for all of the tandem acrocentric tests made. The formula for* c *is given in text. The letters in parenthesis indicate the source of the data. T.A. = tandem acrocentric,* $X\text{-}Y = YSX\cdot YL$, y B

Cross	4(Pat. ♂♂)	4(T. A.)	Single chromosomes	c
T.A./Y × X-Y/0 (A)	3,628	1,088	1,214	0.92
T.A./0 × X-Y/0 (B)	2,748	832	852	0.80
T.A./0 × X-Y/FR2 (C)	8,452	2,320	1,755	0.40
T.A./0 × X-Y/0 (D)	7,824	2,244	1,714	0.44

Before analyzing the data, it is necessary to consider one point. It can be seen from the data given in tables 8 and 9 that sets B and D, which are given as genetically identical (although, indeed, sets A and B were done considerably earlier than sets C and D), do not, in fact, agree at all. Moreover, set B is itself suspect because there were many fewer rod-bearing females recovered than rod-bearing males, whereas in the other three sets there is the expected equality. Consequently, the results from set B will not be considered in the following discussion.

If set A (in which the compound-bearing females carried a Y chromosome) is compared with sets C and D (in which the parental females carried no homolog), it is

TABLE 11

The ratio of single rod X chromosomes recovered as females to patroclinous males, the ratio of rod X chromosomes recovered as males to patroclinous males, and the ratio of tandem acrocentric compounds to patroclinous males, for all of the tandem acrocentric tests recorded. T.A. = tandem acrocentric; $X\text{-}Y = YSX\cdot YL$, y B. *The letters in parenthesis refer to the source of the data*

Cross	Rod ♀♀/Pat.♂♂	Rod ♂♂/Pat. ♂♂	T.A. ♀♀ /Pat. ♂♂
T.A./Y × X-Y/0 (A)	0.68	0.66	0.30
T.A./0 × X-Y/0 (B)	0.57	0.67	0.30
T.A./0 × X-Y/FR2 (C)	0.43	0.40	0.27
T.A./0 × X-Y/0 (D)	0.43	0.45	0.29

evident that there is a reduction in the recovery of single rod-bearing progeny. This reduction can be seen more clearly by reference to table 11 in which the ratios of compound females to patroclinous males, rod-bearing females to patroclinous males, and rod-bearing males to patroclinous males are given. This reduction in the recovery of newly generated single chromosomes by the tandem acrocentric in the absence of a homolog parallels the reduction, noted in the last section, of recovered single rings from tandem metacentric and tandem ring compound X chromosomes without a homolog.

DISCUSSION

It has been shown above that females carrying tandem compound X chromosomes without a homolog (a Y chromosome) produce proportionately fewer offspring carrying newly generated single chromosomes than do such females with a Y chromosome. The three compounds which have been considered, the tandem metacentric, the tandem acrocentric, and the tandem ring represent all of the compound X chromosomes which regularly generate single chromosomes by crossing over.

In order to make the results from the experiments differing by the presence or absence of the Y chromosome agree with each other, it is necessary to assume that approximately 25 percent of all new single rings from the tandem metacentric are not recovered when there is no Y chromosome present in the parental female, and that about 40 to 50 percent of single rods generated by the tandem acrocentric in the absence of a homolog are not recovered.

Determining the exact cause of a discrepancy of this sort is more difficult in the case of the compound chromosomes than free X chromosomes because of the complexity of the genetic situation. One might imagine, for instance, that this discrepancy could arise as a consequence of a shift in exchange frequencies depending upon whether or not a homolog was present. This, as well as a change in the degree of non-randomness, has been considered in the text and has been found not to be compatible with the data. Without going into detail here, it may be said that other deviations from normal meiosis have likewise been examined and found wanting. These deviations include the possibility of chromatid interference, an equational rather than reductional first division (in any proportion), a difference in the behavior of anaphase II bridges, misdivision of the centromeres and strand preference. The simplest interpretation of the genetic results appears to be that some fraction of the single chromosomes newly generated in the absence of a homolog are not recovered in the progeny. At the present time it is not at all clear why a considerable proportion of single chromosomes newly generated by compounds should be lost; it is conceivable, for instance, that chromosome reduplication and exchange in these compounds proceeds abnormally when there is no homolog present, but thoughts along this line are speculative.

In the light of this observation, it is clear why certain tandem acrocentric tests (NOVITSKI 1954; sets C and D, table 10) give no evidence for non-randomness. In fact, it was not realized that the parental females used in the initial test (NOVITSKI 1954) did not carry a Y chromosome in some 80 percent of all cases until this effect of the Y chromosome on the recovery of the single chromosomes became apparent and the pedigree for those crosses was rechecked. When the tandem acrocentric carries

a homolog, however, the c value is extremely high (0.92). This is not an entirely unexpected result. Single exchanges in the tandem acrocentric compound are of two distinct types which presumably occur equally frequently. The first of these types produces an asymmetric dyad composed of a single rod and a tandem acrocentric compound. The other type produces a dyad composed of a single rod and a compound composed of three X chromosomes. The equation given above for c assumes that there is the same amount of non-randomness in both types of dyad. It may very well be, however, that there is in fact a much greater degree of non-randomness in those dyads in which a single chromosome separates from a triple (perhaps even approaching 100 percent) than in the case in which the single chromosome separates from the double.

SUMMARY

Tests of females carrying tandem metacentric or tandem acrocentric compound X chromosomes, with and without a homolog (the Y chromosome) have been made. It has been found that in the absence of a homolog, there is a deficiency of recovered single chromosomes newly generated by crossing over within the compounds. This same effect is manifest in previously published data on the tandem compound ring X chromosome. This deficiency of single chromosomes is greater for the case of the tandem acrocentric than for the case of the tandem metacentric. (It is not, as yet, possible to measure it in the tandem ring.) Various possible reasons for this reduced recovery of single chromosomes are considered. The simplest interpretation of the data seems to be that some fraction of the newly generated chromosomes are lethal when the compound bearing female does not carry a homolog; it is not yet possible, however, to explain why such chromosomes should be lethal.

These results explain why certain tandem acrocentric tests do not give evidence for non-random disjunction. The non-random effect (which is a recovery of single chromosomes over expectation) is masked by the reduced recovery of single rods in the absence of a Y chromosome. When a Y chromosome is present in the parental female, the tandem acrocentric compound shows a high degree of non-randomness ($c=0.92$).

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A COMPARISON OF GENETIC AND ENVIRONMENTAL INFLUENCES ON THE SHAPE OF THE AXIS IN MICE

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BIOMETRICAL analyses of the regulation of development have necessarily dealt with the relationships between anatomical parts. Most of these attempts have remained in the realm of allometry, the principles of which were developed and elaborated upon by HUXLEY (1932) and others (see BONÉ 1955; and BERRILL 1955). However these studies have failed to establish a universality of the allometry equation since many exceptions to a strict adherence of data to the hypothesized straight-line relationships have been noted (see RICHARDS and KAVANAGH 1945; and REEVE and HUXLEY 1945). Nevertheless, allometric methods still provide a useful descriptive tool.

Genetic studies of this problem have been concerned largely with comparisons of body proportions of animals differing in one or more major genes, the number of genes being either known or hypothesized (see PECK and SAWIN 1950).

An altogether different approach to the problem of regulation of development was made by WRIGHT (1932, 1954) who relegated the relationships of sizes of parts in the rabbit and the fowl to a hierarchical grouping of general and special factors. In the same study he roughly estimated the relative contributions of genetic and environmental factors. BURT (1943) has also used the methods of factor analysis on human physical data.

Other than in the study made by WRIGHT (cited above), differences in the roles played by the environment and the genome have especially been lacking. Usually these sources of influence are compounded in analyses such as the studies of allometry—one possible answer for the deviations from the straight-line relationship. Consequentially important questions left unanswered are: How do the environmental and genetic forces differ in their control of relative growth and where do environment and heredity meet in their shaping of an organ during development? Does the environment act through or upon developmental pathways, augmenting or diminishing gene action as suggested by GOLDSCHMIDT (1938)?

Also wanting in these biometric studies are analyses dealing with single organs such that the relationships of the adjacent parts and the shape of the organ are analysed. However, a descriptive analysis along this line was made by BATEMAN (1954) in which he made use of two inherited skeletal anomalies for deciphering the shape changes of individual bones occurring during early development in the mouse. The present study is largely concerned with the latter two problems, i.e. (1) the differences and interrelationships between the environmental and genetic effects and (2) the properties of the size relationships between parts of a single bone.

With the premise that the shape of an organ may be regarded as the resultant of

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the many forces of the genome, the environment and their interactions, the following study is an attempt to analyse some of the influences of these forces upon the shape of the axis. This has required the analysis of the forces into three portions or sources of variance—inter-strain (genetic), inter-litter (environmental) and intra-litter (environmental)—for a number of shape-defining dimensions. The relationships between these various dimensions were then analysed for each of these sources of variance. (The genetic-environmental interaction portion of variance has been assumed to be negligible in this study.)

METHODS

Skeletons of adult, 90-day old male mice were prepared by the papain-digestion technique (LUTHER 1949, quoted by BATEMAN 1954). Each of 18 strains was represented by six animals (except the N/Blk strain having but four). Intra-strain grouping of the six animals into three subgroups, two mice per subgroup, was made on the basis of (1) their being littermates or (2) their possessing a common time of birth but not coming from the same mother. It was regretted that littermates could not have been the sole basis for this subgrouping. However the only efficient means of collecting representative groups of the 18 strains in a reasonable period of time made this procedure necessary. On this account, in the analyses below the variances and correlation coefficients termed "intra-litter" will contain an unknown portion of the inter-litter variance.

The 18 strains used are shown in table 1 with their inbreeding histories. With the exception of the Large strain, all are inbred at least to the point of F being 0.90.

TABLE 1

Inbreeding histories of the strains used in the experiment

Strain	Generations B-S inbred
A/Jax	89
Ake	14
BALB/Jax	90
CBA	84
C57BL/6	39
C3H/Ks	14+
DBA/My	20+
Large (GOODALE)	3*
LP	42
ND	30
129	14+
RIII	16
Small (MACARTHUR)	11
S/Emv	19
ST	39
Swiss	19
SWR	42
N/Blk	15

* Previous closed-line mating system brings estimate to $F = .60$ (personal communication from DR. CHAI).

An estimate of the maximum average genetic variance remaining within all 18 strains, is three percent, a very small amount. Any genetic variance or covariance that might remain within strains would be included in the inter- and intra-litter portions. This contamination would tend to dilute any differences found between the genetic and environmental kinds of variances and correlation coefficients.

In this study only the axis and the innominate bone were used. The innominate length rather than body weight was selected to serve as a standard of general skeletal size, for body weight is more subject to everyday environmental influences and it involves all kinds of tissues. Measurements were made on camera-lucida outlines of the posterior views of the axis. The innominate was measured directly by a vernier caliper.

The several measurements are shown in figure 1 as:

AH—arch height, distance from mid-point of dorsal surface of centrum to mid-point of ventral surface of arch.

AW—arch width, distance between medial surfaces of arch at dorsal edges of articulating surfaces.

L—lamina length, distance from mid-point of ventral surface of arch to medial surface of arch at dorsal edge of articulating surface.

P—pedicle length, distance from line measuring transverse process length perpendicularly to medial surface of arch at dorsal edge of articulating surface.

T—transverse process length, distance from dorsal corner of transverse process tip to mid-point of line drawn from dorsal to ventral centrum-base junctions.

B—base length, distance from point where pedicle meets line of transverse proc-

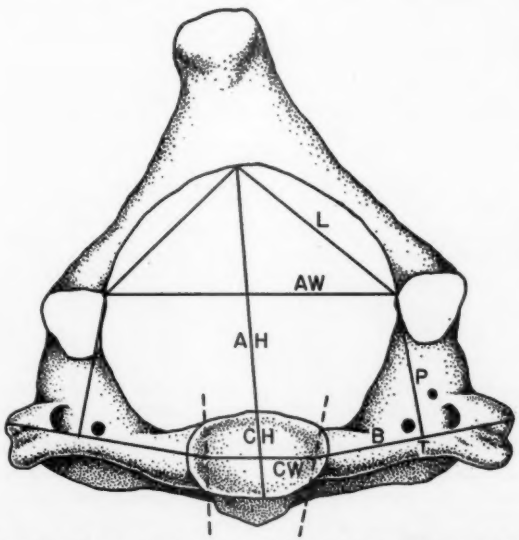

FIGURE 1.—Drawing of posterior view of the axis showing dimensions used in the analysis.

ess measurement to mid-point of line drawn from dorsal to ventral centrum-base junctions.

CH—centrum height, distance from mid-dorsal to mid-ventral surfaces of centrum.

CW—centrum width, distance between mid-points of a line drawn from dorsal to ventral centrum-base junctions.

I—innominate length, distance of longest dimension measured from crest of ilium to caudal edge of the pubis.

Right and left measurements of bilateral dimensions (except the innominate) were combined for more accurate measurement. The mean values and subsequent calculations of these bilateral measurements are based upon these "doubled" values except where otherwise noted. Each unit of measure is equal to 0.0513 mm for the axis and to 0.1 mm for the innominate.

RESULTS

Variance

The components of variance for each of the measurements and for each of the sources of variance (inter-strain, inter- and intra-litter) are shown in table 2. Those variances which were of statistical significance when tested in an analysis of variance are indicated. All of the inter-strain variances are shown to be of high statistical significance. This is not surprising, for strains of such diverse origin and maintained under such close inbreeding as the majority of the ones used here would be expected to have quite diverse mean measurements if heredity were at all important.

The inter-litter variances, on the other hand, are of statistical significance for only a few of the dimensions. It is seen that the dimensions which are of significance deal with laterad and associated measurements.

The differences in variability are more easily comparable in table 3 where the coefficients of variation are presented for each dimension and for each source of

TABLE 2

Estimates of the components of variance

Dimension	Source			Heritability estimate
	Inter-strain	Inter-litter	Intra-litter	
AH	2.84**	0.31	2.73	.318 ± .098
AW	4.72**	0.65*	1.89	.482 ± .089
L	10.24**	0.00†	6.64	.435 ± .121
P	11.11**	5.29**	5.26	.344 ± .072
T	14.48**	1.85*	5.06	.512 ± .088
B	11.44**	3.53*	6.89	.354 ± .081
CH	1.62**	0.10	0.59	.539 ± .095
CW	4.01**	0.91*	1.83	.425 ± .084
I	115.90**	9.29*	10.26	.748 ± .052

* Significant at the 5% level.

** Significant at the 1% level.

† Intra-litter mean square greater than inter-litter mean square.

TABLE 3

Means for each measurement with coefficients of variation arranged according to source of control (inter-strain, inter-litter and intra-litter). The figures in parentheses are rank orders of the variabilities from low to high and of the means from high to low within each source

Dimension	Source								Mean**	
	Inter-strain		Inter-litter		Intra-litter		Total			
AH	4.33	(2)	1.43	(2)	4.25	(5)	6.23	(3)	38.906	(2)
AW	4.27	(1)	1.58	(3)	2.70	(2)	5.30	(1)	50.868	(5)
L	4.76	(3)	0.00*	(1)	3.84	(4)	6.12	(2)	67.198	(3)
P	8.40	(8)	5.80	(9)	5.78	(7)	11.74	(7)	39.689	(6)
T	6.25	(5)	2.23	(6)	3.69	(3)	7.59	(5)	60.915	(4)
B	6.47	(6)	4.90	(8)	6.85	(9)	12.21	(8)	38.330	(7)
CH	8.17	(7)	2.04	(5)	4.92	(6)	9.76	(6)	15.576	(8)
CW	9.43	(9)	4.48	(7)	6.36	(8)	12.23	(9)	21.245	(9)
I	5.98	(4)	1.69	(4)	1.78	(1)	6.46	(4)	179.915	(1)

* Intra-litter mean square greater than inter-litter mean square.

** Bilateral measurements (see text) have been reconverted to "single" values in computing rank orders of means.

variation. There is a considerable diversity among the dimensions in their variabilities. All three sources of variance show similar rankings of the dimensions in regard to the magnitudes of the variabilities.

Heritabilities

Estimates of the heritabilities of the dimensions were made on the assumption of the strains having been developed as unselected lines from a common panmictic population, and the present strains having been inbred an infinite number of generations. On an additive scheme, heritability will be equal to:

$$\frac{\sigma_S^2}{\sigma_S^2 + 2\sigma_L^2 + 2\sigma_I^2}$$

where σ_S^2 is the inter-strain variance, σ_L^2 is the inter-litter variance and σ_I^2 is the intra-litter or individual variance. The estimates are shown in table 2.

Heritability usually refers to the proportion of the variability in a random bred population that is genetic; here it is the genetic component in an array of closely inbred lines. Only if all gene action were additive would the two be equivalent.

Correlations

The interrelationships of the dimensions are shown in tables 4, 5 and 6 as correlation coefficients. Those coefficients which individually show a statistically significant difference from zero are indicated. The correlation coefficients were computed from their respective mean squares and products for the intra- and inter-litter sources and from the variance and covariance components for the inter-strain source. Since the inter-litter mean square for L (lamina length) was less than the intra-litter mean square, it was necessary to use the mean squares and product to compute the inter-

TABLE 4

Inter-strain correlation coefficients between the various measurements of the axis and the innominate

Measurement	AH	AW	L	P	T	B	CH	CW
AW	.05							
L	.58*	.73*						
P	.01	−.30	−.41					
T	.20	.27	.16	−.07				
B	−.06	.54*	.33	−.68*	.39			
CH	.07	−.37	−.22	.59*	.12	−.84*		
CW	.09	.20	.17	.05	.04	−.41	.63*	
I	.36	−.22	.00	.22	.46	−.28	.73*	.53*

* Significantly different from zero at the 5% level.

TABLE 5

Inter-litter correlation coefficients between the various measurements of the axis and the innominate

Measurement	AH	AW	L	P	T	B	CH	CW
AW	−.11							
L	.30	.55*						
P	.31	.20	−.13					
T	.35*	.09	.20	.06				
B	.28	−.03	−.14	−.25	.50*			
CH	.04	.26	.26	.24	.02	−.39*		
CW	−.19	.41*	.32	.19	−.41*	−.63*	.39*	
I	−.21	.28	.42*	.00	−.07	−.50*	.44*	.53*

* Significantly different from zero at the 5% level.

TABLE 6

Intra-litter correlation coefficients between the various measurements of the axis and the innominate

Measurement	AH	AW	L	P	T	B	CH	CW
AW	.36*							
L	.74*	.54*						
P	.27	−.07	−.13					
T	.22	.04	.13	−.06				
B	.24	.34*	.00	−.13	.32*			
CH	.11	.05	.19	.15	−.04	−.05		
CW	−.02	.37*	.22	−.19	−.29*	−.36*	.23	
I	.04	.15	.14	−.02	.23	−.02	.13	.04

* Significantly different from zero at the 5% level.

litter coefficient of the lamina. This procedure was carried out for the other dimensions as well, in order to be consistent. Actually the use of the components of variance and covariance gave but little change in the values derived from the mean squares and products. (It has been assumed here that the inter-litter correlations do not differ

TABLE 7

Tests of homogeneity of correlation coefficients

Source	d.f.	χ^2	*P*
Within sets:			
Inter-strain	35	91.07	<.01
Inter-litter	35	106.42	<.01
Intra-litter	35	120.64	<.01
Between sets:			
Inter-strain			
Inter-litter	36	40.23	.30 to .35
Inter-strain			
Intra-litter	36	46.28	.10 to .15
Inter-litter			
Intra-litter	36	14.73	.95 to 1.00

between strains nor that the intra-litter correlations differ between litters other than by chance.)

Tests for homogeneity (see RAO 1952, p. 233) of the three sets of correlation coefficients are summarized in table 7. There is strong evidence that the coefficients are heterogeneous within the sets ($P < .01$). On the other hand, when the coefficients are tested between sets, there is no evidence that corresponding coefficients are different from one another. This latter finding made desirable further inquiry into the possible existence of more subtle differences between these sets of coefficients than was demonstrable by the tests of homogeneity.

Correlated responses to "selection"

The present data were not of a high enough level of reliability to warrant the use of such techniques as factor analysis or WRIGHT'S method of hierarchical grouping. Therefore, the following procedure was devised in order to uncover what consistent trends in differences between the three sets of correlation coefficients the array of coefficients may offer.

Each dimension in its turn was arbitrarily increased by 30 percent of its mean measurement and the theoretical correlated responses for all the other dimensions were calculated. This procedure is an elaboration of an equation concerning correlated genetic responses to artificial selection presented by LERNER (1950), p. 236. Thus, each dimension increased provides the resulting theoretical change in all other dimensions and thus in shape of the whole vertebra as shown diagrammatically in figure 2. The same procedure has been carried out for the environmental correlation coefficients as well as the genetic, a procedure admittedly lacking in much biological meaning and practical use other than serving for comparison purposes in the present study.

The procedure has the advantages of condensing the information from several dimensions into a single diagram indicating shape change and of supplementing the information derived from the correlation coefficients, with the computed variances.

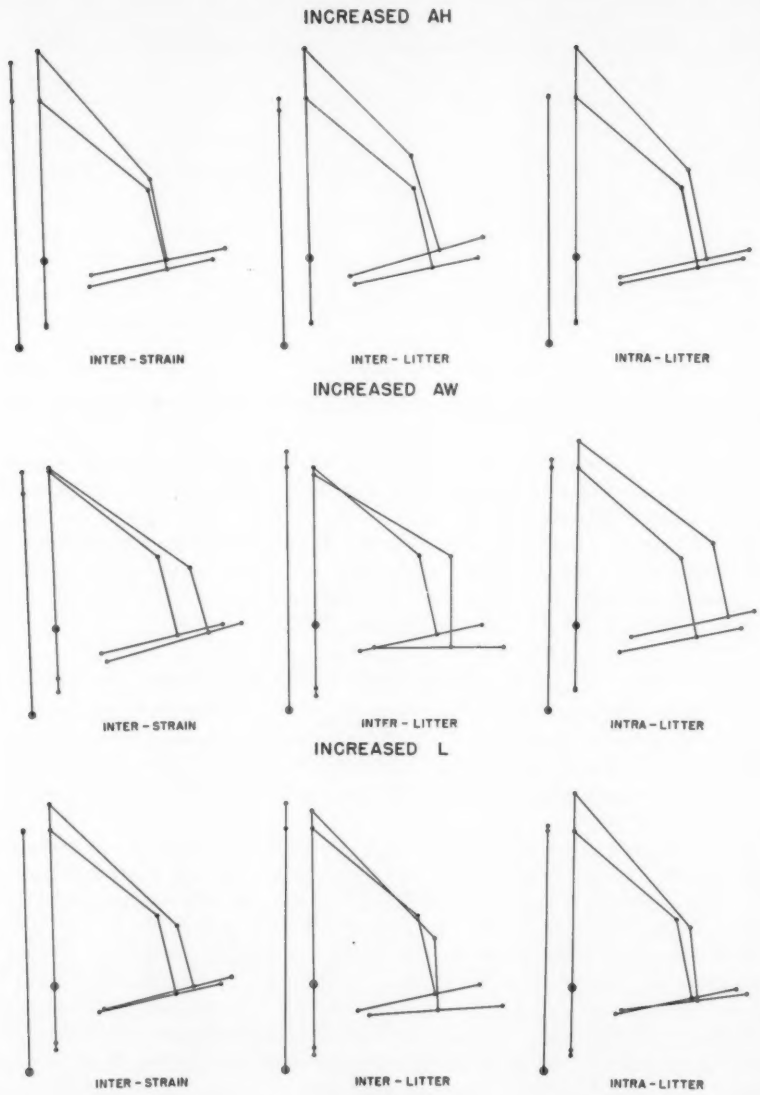

FIG. 2a.

FIGURE 2.—Diagrams of change in shape of the axis due to increase by 30 percent of the indicated dimension. Only halves of vertebrae are shown. A line along side the vertebral half represents the innominate length. The standard (average) vertebral shape and innominate length are indicated by ●——●, whereas the altered vertebral shape and innominate length are indicated by ○——○.

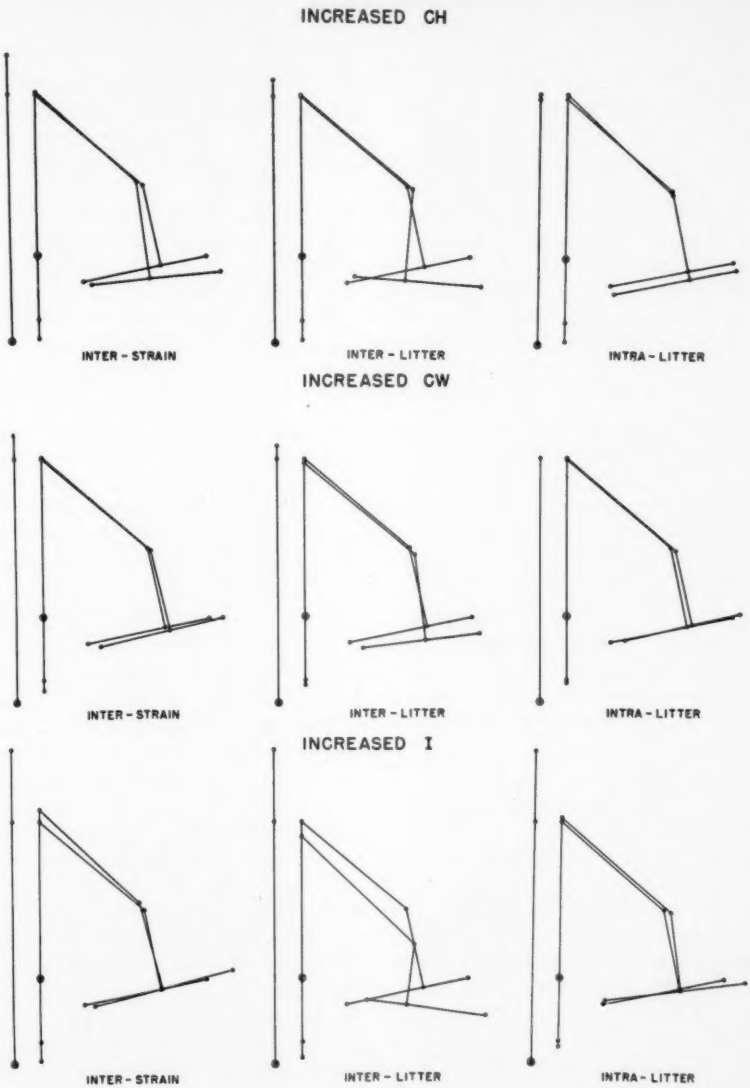

FIG. 2b.

However, the procedure provides no means of statistically evaluating the evidence obtained; only tendencies may be described.

The diagrams in figure 2 represent the theoretical changes in shape of the vertebra due to the effects of the increase in the specified dimension upon all the other dimen-

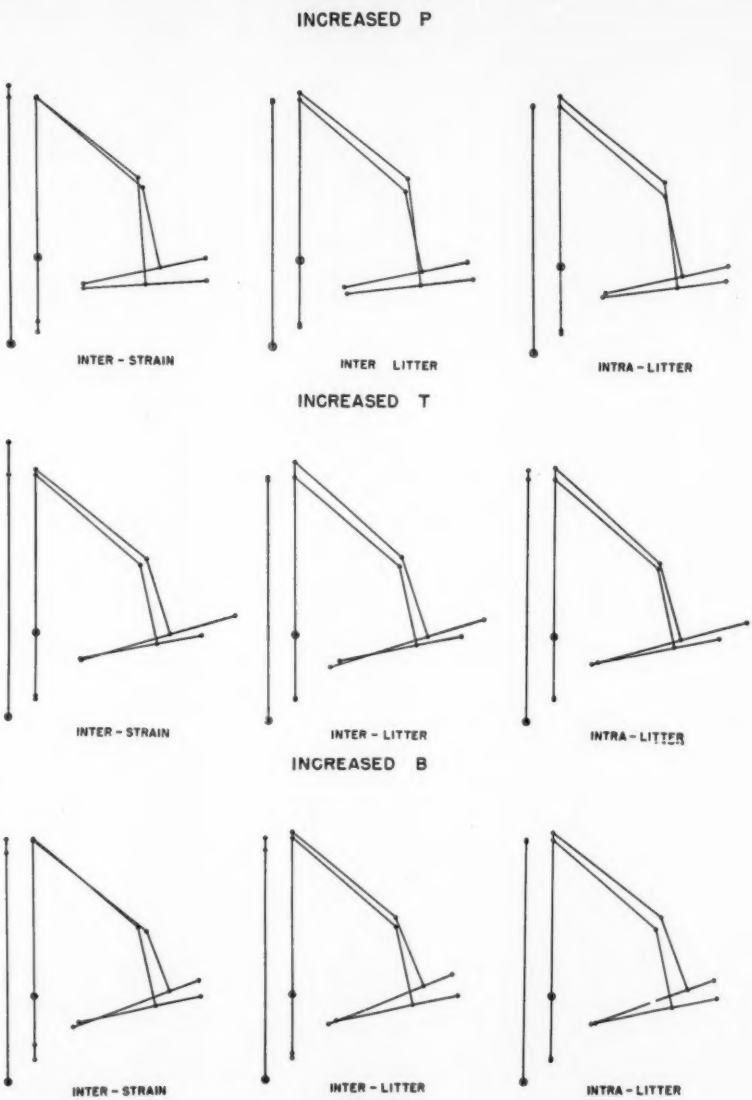

FIG. 2c.

sions. Only half of a vertebra is depicted. All distances are reconstructed in reference to the "unselected" average vertebra. The mid-dorsal point of the centrum is the common reference point for each diagram. A line, with its reference point below, is also drawn along side the vertebra half to show the relative changes in the innominate length.

DISCUSSION

Variance

It is reasonable to expect certain anatomical parts to be more or less variable than others. Developmental systems may involve pathways more or less affected by either the genetic background or the environment. The degree of variability brought about by outside agents may be due to adaptive physiological arrangements built up by natural selection for a more efficient system of development, or it may be due to the intrinsic degree of stability of the physico-chemical systems involved. In this sense, an increase in the variability of a tissue may be due to an adaptive change of the tissue under observation (e.g. muscular hypertrophy in response to exercise) or it may be due to an instability of the tissue which lacks means to adjust to the particular environmental stresses present (e.g. the non-regenerating mammalian limb). On the other hand, a decrease in the variability may be due to adaptive changes in the regulatory processes of the substance under observation (e.g. the homeostatic regulation of the blood sugar or blood pH level) or it may be due to an intrinsic stability of the observed tissue toward the particular environmental stresses present. It would be most difficult to distinguish between adaptive and intrinsic causes of variability or to determine the degrees to which both kinds are present.

Another explanation for differences in variability is that some developmental systems may involve a greater or lesser number of pathways—along with their various interactions—and thereby may allow a greater or lesser chance for outside agents to upset the normal course of events. This would be most evident when several functionally and developmentally distinct tissues are in conjunction.

In table 3 all three sources of variance show the higher variabilities for the centrum and nearby measurements (CW, CH, P and B), whereas the neural arch measurements (AW, AH and L), essentially neural tube dimensions, tend to be the least variable. It may be that a greater stability of the latter tissue is essential to the organism. The more variable measurements are those which could no doubt be influenced by developmental systems of different tissues, viz. neural, muscular and skeletal. These kinds of tissues would also be expected to possess more adaptive flexibility to cope with outside influences. These general areas of differences in variabilities may be seen below to be associated with the changes in shape of the vertebra.

In the present data there is an indication of a negative relationship existing between the variability and the size of the means. This is especially evident after the means of the bilateral measurements have been halved. This relationship is noticeable in the ranking presented in table 3. This relationship, however, may be a spurious one and due to the fact that the larger dimensions are concerned with arch measurements while the smaller dimensions are concerned with the centrum and adjacent measurements. Thereby the differences in variability may be intrinsic to the tissues involved (above) and not necessarily related to the size of the dimension.

The heritability estimates are comparable to heritabilities found for body weights in mice (FALCONER 1953). One exception in the present case is the unusually high value for the innominate measurement. The innominate measurement is exceptional in that it probably is highly correlated with body weight, for the Large strain showed an overly long innominate (deviation $>2\sigma$). This one value in the calculation of the between-strains variance might well have unduly increased the genetic variance

estimate for the innominate in comparison to the other values. The Small strain however did not show an exceptionally small innominate.

The genetic variances used to compute the estimates of heritability were derived from the between-strain variance which would possess non-additive as well as additive gene effects. Therefore the computed heritabilities would be larger than those based on correlation between relatives in randomly mating populations.

There is no particular pattern of areas of the axis possessing high heritabilities as was observed for the variabilities above. Also the heritabilities show no consistent trend in their relationships to the magnitudes of the total variabilities (coefficients of variation) of the dimensions. This means that according to these data there is just as much genetic control over the total variability of a dimension which is greatly variable as over that which is but little variable. This is one outcome which concurs with the finding above that the magnitudes of the variabilities of the dimensions are generally correlated between the three sources of variance.

Correlations

The simultaneous change in one dimension of an organ with that of another dimension may be due, other than to chance, to any of several causes, genetic or environmental. Genetic correlations may be due to pleiotropy (common genes active in the development of both dimensions) or may be due to linkage (not likely in the present case). Pleiotropy may be effective by biochemical similarities (e.g. developmentally through like competences of the two tissue masses) or by mechanical associations, (e.g. one tissue part passively altered by the active growth of an adjacent part).

In relative growth studies, positive relationships seem to be the usual finding. This is due to the fact that the data usually involve actively growing parts. Growth, of course, tends to make strong positive correlations or regressions. Also the measurements used in most of the studies on relative growth and those measurements used by WRIGHT are of body parts remotely connected, i.e. body length, humerus length, femur length, etc. and sometimes of adjacent bones, i.e. ulna, radius, etc. but usually these are compared to general body size and not to each other. Measurements on the same organ seem to have been neglected. The present study involves several measurements on the same bone which may well account for the discrepancy found here with other studies in regard to the sign of the coefficients.

In the present study, several negative correlations were observed. The most likely explanations for the negative relationships are that active growth is not compounded with the other sources of variance present and that adjacent parts are involved in the measurements, one part passively submitting to active growth of the other part during development, e.g. the lamina-pedicle relationship. Even if two parts are not adjacent, themselves, a measurement strongly correlated with a distant measurement which is in turn adjacent to a third measurement, may be negatively correlated with the third measurement, e.g. base length-centrum height relationship, where the centrum height is positively correlated with the centrum width, which is adjacent to the base length. These negative correlations, however, do not preclude the general size factors of CASTLE, WRIGHT and others, so often hypothesized.

The significant negative genetic correlations are found in those relationships of base length. It is reasonable to suppose this dimension is passively controlled. It

can be considered a function of centrum width, arch width and slope of transverse process (see fig. 1).

Environmental correlations may be due to a common reaction of different genetic systems to the same environmental change (common environment) or common genetic pathways of two or more dimensions effected by environmental stimuli of all sorts. The latter kind would tend to simulate genetic relationships for the traits involved, as perhaps they do in the present study.

The homogeneity between sets of correlation coefficients may be due to residual genetic variance, to the environment acting generally upon the genetic pathways involved, to an insensitive test, i.e. there are differences but they are not shown to be significant. None of these are mutually exclusive.

Although the amount of genetic variance remaining within strains due to using some low-inbred strains is estimated to be not greater than three percent, a greater genetic variance than expected may remain within strains due to natural selection of the heterozygotes in opposition to the pressures of inbreeding. Residual genetic variance can never be fully ruled out as the cause of the homogeneity between sets, however it is doubtful much genetic variance remains within the strains since such strong genetic variance between strains is indicated.

On the other hand, the finding of possible homogeneity between environmental and genetic sets of correlations may be interpreted to mean that the environment is acting upon the developmental pathways at time of and in a manner similar to that of gene action. This is as might be expected; the genetic pathways may well be proven to be the framework upon which the environment has its main effects. This interpretation is further strengthened by the observed relationship between the rankings of the magnitudes of the variabilities of the dimensions between the three sources (table 3). These similarities indicate common origins of the variabilities among the different sources.

The fact that the environment can simulate genetic traits has long been recognized in experiments producing phenocopies (GOLDSCHMIDT 1936, and others). Developmental defects in mammals which resemble genetic diseases have also been produced by stresses invoked upon the pregnant mother (see RUNNER 1954). Just how closely these cases tie in with the present study is merely speculative at present. However, the anomalies which are induced by stress situations on the pregnant mother, as well as the expressed phenocopy, were shown to be dependent upon the genotype. This dependency was further shown by WADDINGTON (1952) in his selection experiments in Drosophila. Thus, the genetic pathways must be present for the environmental effect to be brought to the forefront, just as in the present case the environment appears to bring forth the existing genetic background expressible in axis shape.

Correlated responses to "selection"

In the diagrams of figure 2 these consistent trends may be seen:

Individual (intra-litter)

(1) Arch dimensions do not change independently of one another.
(2) All other dimensions do change independently of one another.

Litter (inter-litter)

(1) Arch dimensions do change independently of one another.
(2) Centrum dimensions do not change independently of one another.
(3) A close relationship exists between the innominate length, centrum dimensions and the bending of the articulating surfaces and the transverse processes.

Strain (inter-strain)

(1) Arch dimensions do change independently of one another.
(2) Centrum dimensions do not change independently of one another.
(3) A relationship exists between the innominate length and the centrum and the innominate length and the length of the transverse process.

No doubt other tendencies may be found, however the consistency of the above noted trends and their non-random nature leads to the conclusion that differences as well as similarities may exist in the ways by which these three sources of control effect the shape of the vertebra.

The most striking difference in the influences on shape of the three sources of control, is the strong effect which the inter-litter environment has upon the innominate length, ventral tipping of the articulating surfaces and the transverse processes (fig. 2). It is difficult to conceive of a strong direct embryonic relationship between the two regions of the innominate and the cervical vertebrae. The powerful inducing effect which the innominate seems to have on the transverse processes of the sacral vertebrae would hardly be considered in operation at this distance on the transverse processes of the cervical vertebrae, for the sacral inducing effect has a relatively sharp threshold anteriorly of the sacrum.

Probably a more acceptable explanation for this inter-litter effect is suggested by the strong correlation of both the innominate and axis shape with the degree of general development. BATEMAN (1954) has shown how the shape of the axis changes during its early development. The axis which he has drawn (p. 223) shows a considerable lowering of the articulating and transverse processes as the vertebra develops. The length of the innominate shows a substantial increase during this same period. Thus if the inter-litter environment is essentially augmenting the progress of development we would observe the picture somewhat as that shown in figure 2 where the innominate has been arbitrarily increased by 30 percent. Interference with developmental progress would show the opposite effect.

The explanation which is tentatively most able to fit the picture of environmental action as coming from inter-litter sources, then, is that the environment acts rather closely with developmentally-early gene action, enhancing or depressing the genetically controlled processes, thus providing very similar correlation coefficients to those of the genetic source. In addition to the effect on the early processes, this enhancing and depressing effect also influences the degree of general development of the organism as a whole, thereby determining to what extent development will progress, providing the subtle effects seen in figure 2.

The lesser alterations of axis shape in figure 2 under the influences of the intra-litter environment are perhaps due to the lesser severity of and different kind of environ-

mental effects on individuals within the litter. Perhaps a large proportion of the effects seen in the intra-litter portion is also due to errors of the human kind in making the original measurements.

Effects on Selection

If the tentative conclusion reached from these data is true, i.e., that the environmental effects simulate gene action in morphogenesis, then the process which WADDINGTON (1952) has described as "selection of the genetic basis for an acquired character" may be a more common type of selection than previously supposed. If the environment aids the population to explore other genetically possible forms than represented in the population's array of genotypes evolutionary progress would be much enhanced. This would be tantamount to increasing the genotypic range or the unmasking of extreme forms which would ultimately be genetically attainable through selection. This possibility also suggests that a multiplicity of environments would provide the animal breeder in his selection program with a broader basis of evaluating the possible-attainable genotypes in his present animal population.

Such a possible effect is admittedly contrary to the concept of environmental hindrance in evolutionary and animal breeding processes. On the other hand, it is difficult to find an investigation where the environment has been directly examined in regard to its effect on genotypic range. The question of the effects of selection under varied environments has been frequently discussed (see FALCONER 1952). The present suggestion is concerned however, with a multiplicity of environmental conditions rather than an optimal one. A strong environment-genotype interaction would, of course, interfere with such a process and certainly limit its effectiveness. However, the present data suggest the existence of very little of this type of interaction for such an interaction would be expected to decrease the homogeneity between the sets of correlation coefficients.

SUMMARY

The axis and innominate bones were collected from mice of 18 inbred strains. Measurements of several dimensions of the axis as well as the innominate length were made to furnish the data required in the analysis of the genetic and environmental influences on the shape of the axis.

The analyses of variance and covariance provided information for estimating variabilities, heritabilities and intercorrelations of the dimensions separately for each source of control, i.e. inter-strain, inter-litter and intra-litter.

The inter-strain variances (genetic) were highly significant for all dimensions.

The inter-litter variances (environmental) that were significant were localized to laterad and associated measurements.

Rankings of the magnitudes of the variances of the dimensions were similar for the genetic and environmental sources.

The centrum and adjacent measurements had the higher variabilities, the neural arch measurements had the lower variabilities.

Homogeneity tests of the correlation coefficients indicated that coefficients within a set (source of control) were heterogenous but that corresponding coefficients be-

tween sets showed no evidence of being heterogeneous. This indicated a strong similarity between environmental and genetic correlation coefficients.

Theoretical responses of the axis shape to "selection" (arbitrary increase of a dimension) were diagrammed and consistent trends were indicated.

The possible relationships between environmental effects and gene action with their consequences for selection, natural and artificial, were discussed.

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INTERACTIONS BETWEEN SOME RECESSIVE LETHAL AND VISIBLE MUTATIONS OF THE FOURTH CHROMOSOME OF DROSOPHILA MELANOGASTER

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EXPERIMENTAL populations of *Drosophila melanogaster* kept by DR. BRUCE WALLACE and his collaborators (1951) received chronic radiation of approximately 2,000r per generation. The effect on the fourth chromosome was analyzed by these investigators by outcrossing males from the irradiated populations with females carrying the dominant mutations, "cubitus interruptus" (ci^D) and "eyeless" (ey^D) as markers. Male progeny were again backcrossed to ci^D/ey^D females. From the progeny of this cross, males and females carrying the same dominant markers and the fourth chromosome being tested, were then intercrossed. If these progeny contained no wild type flies, the fourth chromosome in question must have been lethal in double dose. By this method 13 different fourth chromosomes which were lethal when homozygous were obtained from two experimental populations.

It was assumed because of the shortness of the fourth chromosome that it possesses a relatively small number of loci capable of producing lethal or visible mutations. Therefore possibly a large proportion if not all the lethal producing loci might be included among these 13 lethals. Also if a single locus is capable of producing both recessive lethal alleles and visible alleles, then again because of the shortness of the chromosome, some of the known visible mutations of the fourth chromosome might prove to be alleles of some of the 13 lethals.

In order to investigate these above possibilities the allelism within the lethals was studied. Also studied were the interactions of these lethals with some of the visible fourth chromosome mutations.

PROCEDURE

Eleven of the thirteen lethals were maintained as balanced lethal stocks with ey^D; two were balanced with ci^D. In order to study allelism all thirteen were crossed in all possible paired combinations (78 such combinations in all). Two lethals were considered allelic when no wild type progeny appeared from the cross between them.

In order to study lethal-visible interactions, first a number of fourth chromosome visible mutants were investigated, and two stocks were chosen for study; they were the two balanced lethal stocks, Cataract/cubitus interruptus (Cat/ci^D), and bent/Cubitus interruptus (bt^D/ci^D). The expression of ci^D differed in the two stocks. The ci^D of the bt^D/ci^D stock showed missing segments on wing veins L4 and L5 distal to the posterior crossvein and is hereafter referred to as $ci.^{D^2}$ The ci^D of the Cat/ci^D stock with a piece missing only from L4 is referred to as ci^{D^1}. Each of these two stocks was crossed to all thirteen lethals and the effects of lethal-visible interactions sought among their progeny.

TABLE 1

Allelism tests between the fourth chromosome recessive lethals and four "visible" mutants. Lethals with the same letter behaved as alleles with each other. A minus sign indicates that the heterozygote between the lethal and the "visible" dies; a plus sign indicates that such a heterozygote is viable

Dominant "visibles"	Recessive lethals												
	a-1	*a*-2	*a*-3	*a*-4	*a*-5	*b*-6	*b*-7	*b*-8	*b*-9	*c*-10	*c*-11	*d*-12	*e*-13
Cat	−	−	−	−	−	+	+	+	+	+	+	−	+
bl^D	+	+	+	+	+	−	−	−	−	+	+	+	+
ci^{D2}	−	−	−	−	−	+	+	+	+	+	+	+	+
ci^{D1}	+	+	+	+	+	+	+	+	+	+	+	+	+

RESULTS

The results of the allelism crosses showed that there were only five different loci represented among the thirteen lethals. One lethal locus, designated *a*, was represented five times; another, *b*, four times; another, *c*, was represented twice, and *d* and *e* only once each.

The results of mating the two stocks, bl^D/ci^{D2} and Cat/ci^{D1}, with the lethals are summarized in table 1. The lethals are numbered 1–13 individually and are also designated *a*, *b*, *c*, etc. showing their allelism relationships. In the body of the table, a minus indicates a lethal interaction between the recessive lethal at the head of the column with the visible mutant at the left end of the row. A plus sign indicates no interaction.

The matings of bl^D/ci^{D2} with the lethals revealed the lethal interaction of bl^D with all members of the *b* series. The expected class of bl^D/b, appearing as either bl^D or wild type overlap, was missing from the offspring which were all ci^D phenotypes. This same mating also revealed a lethal interaction between ci^{D2} and all members of the *a* series. The fact that in each case all members of the series interact with a visible mutant in the same way confirms their allelism as determined by lethal interactions with each other.

Matings of Cat/ci^{D1} with the recessive lethals showed a lethal interaction between *Cat* and the *a* series. However, *d* also produced a lethal interaction with *Cat*.

The fact that *Cat* and ci^{D2} both interacted with the *a* series suggested that they might be lethal with each other. Therefore, crosses Cat/ci^{D1} by bl^D/ci^{D2} were made, and it was found that such an interaction took place; the Cat/ci^{D2} class was missing from the progeny. ci^{D1}/ci^{D2} was also lethal.

ci^{D1} was found to be viable with all lethals, including the *a* series with which ci^{D2} produced a lethal interaction. This result adds to the morphological evidence differentiating ci^{D1} from ci^{D2}.

DISCUSSION

The simplest assumption is that when two mutants show lethal interactions when heterozygous, they are alleles. However a simple allelic relationship of "point" mutations will not explain our data. One is forced therefore to more complex hypotheses. As will be seen below there are several such explanations, the differentiation

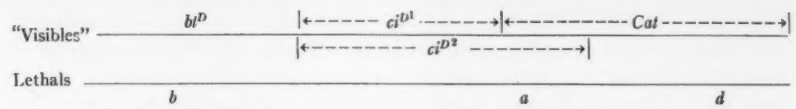

FIGURE 1.—One hypothetical linear arrangement on the fourth chromosome of the lethals and "visibles" studied, assuming deficiencies are involved.

of which requires further work. Unfortunately this necessary additional experimentation cannot be carried out by these authors on this material. Nevertheless, it was considered worth while not only to present the foregoing data as far as they go, but also to consider the several interpretations even though they may be indistinguishable.

First, a system of deficiencies could provide an adequate explanation. Figure 1 is a map showing the relationships of the mutants involved when deficiencies are assumed. BRIDGES and BREHME (1950) state that the ci^D mutation is "possibly a deficiency". Assuming then that our ci^{D1}, is a deficiency, then ci^{D2} would be a deletion of greater length. This would explain the lethal interaction between ci^{D1} and *a*, since *a* might be included in the area of extension by which ci^{D2} exceeds ci^{D1}.

If the ci^{D2} deficiency extended to include *Cat* then the lethal interaction of these two would be explained, as well as the failure of any interaction between *Cat* and ci^{D1}.

The fact that *Cat* is inviable with *d* but that *d* is viable with ci^{D2} and *a* with which *Cat* interacts lethally, could be explained by assuming *Cat* is also a deficiency partially including ci^{D2} but extending to include *d*.

Assuming this system of deficiencies means that bl^D could not be between ci^D and *Cat* since ci^{D2} deficiency includes both ci^D and *Cat*, but not bl^D. This arrangement is at variance with the conclusions of FUNG and STERN (1952) who place bl^D between ci^D and *Cat*. These authors used three deficiencies of the right arm whose left breakage points were close together but essentially unknown, but whose right breakage points could be arranged in a series each farther to the right than the preceding one. As far as this series of right breakage points was concerned the first or shortest deficiency included only *ci*, the second *ci* and *bl* and the third *ci*, *bl*, and *Cat*. (Actually *spa* was used, which is allelic to *Cat*.) Since, however, the left breakage points were unknown there still remains the possibility of several other arrangements including the one presented here.

However, FUNG and STERN's arrangement based on deficiencies is in agreement with the sequence established by STURTEVANT (1952) who used crossover criteria. Furthermore, the system of deficiencies proposed above rests on no cytological evidence. Even BRIDGES' original suggestion that ci^D is a deficiency could not be confirmed cytologically. It seems likely therefore, that some factor other than deficiencies is involved.

An alternative explanation involves the possibility that the two balanced lethal stocks, ci^{D2}/bl^D and ci^{D1}/Cat have accumulated recessive lethals by mutation. Since there is so little crossing over on the fourth chromosome the acquisition of nonallelic recessive lethals on the two chromosomes of a balanced lethal stock is a definite

possibility. If in the ci^{D2}/bl^{D} stock the ci^{D2} chromosome had acquired the *a* lethal and the bl^{D} the *b* lethal, and in the ci^{D1}/Cat stock the *Cat* chromosome had acquired *a* and *d* lethals, then our findings would be explained. That these two stocks should have acquired some of the same recessive lethals which were independently extracted from DR. WALLACE'S populations may seem unlikely, but this likelihood depends on the total number of different loci capable of producing recessive lethals which may be small.

A final explanation of our findings could involve a system of specific interlocus interactions. To us this seems unlikely if only because in general such interactions are the exception rather than the rule. A very special kind of interaction, however, could be involved—namely, position effect. STERN and others (e.g. 1955) have studied, in detail the position effects of the *ci* locus. If chromosomal rearrangements are involved either in our lethal material or the ci^{D} stocks, then our findings could have still another and quite different interpretation.

It should be made clear that these possible explanations (deficiencies, lethals, and interaction) are not mutually exclusive, but a combination of them could be closer to the truth.

Finally it should be pointed out that since there were only five different loci represented among the original 13 independently obtained lethals, there are probably a relatively small number of loci on the fourth chromosome capable of producing recessive lethals. Assuming N loci all with the same mutation rate to lethality, it is possible to write the probability of obtaining 5 different loci in a sample of size 13 as a function of N (LEWONTIN and PROUT 1955). From consideration of this function it is possible to state that the chances are very small that there are more than 20 loci on the fourth chromosome capable of producing recessive lethal alleles.

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STABILITY OF NUCLEAR PROPORTIONS DURING GROWTH OF NEUROSPORA HETEROKARYONS[1]

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AN EXPLANATION for the stability of the normal phenotype which generally results from heterokaryosis between biochemical mutants of *Neurospora crassa* was suggested by BEADLE and COONRADT in 1944. They observed that the growth rate of such heterokaryons on minimal medium equalled that of wild type strains, whereas the proportions of the nuclear types varied widely, even among different heterokaryons composed of the same two mutants. Since the mutants cannot grow separately on minimal medium, it was obvious that at some nuclear ratio outside the observed range, the dosage of the normal allele which is carried only in the nuclei of the minority type would be insufficient to support normal growth of the entire culture. It was surmised that chance fluctuations in the local nuclear ratios might cause different growth rates among individual hyphae. Then, "If selection of rapidly growing hyphae is allowed to go on for some time, one would expect nuclear ratios to adjust themselves so as to give a maximum rate of growth". Thus the failure to observe submaximal growth rates might be explained on the basis of natural selection of nuclear ratios in the optimal range. This idea has become so widely accepted that it was stated (PROUT, *et al.* 1953) without qualification that hyphae with certain nuclear ratios are able to grow faster than others and "will, as a consequence, have a greater chance of forming the frontier of the mycelium and will contribute that favorable ratio of nuclear types to the new growth. Thus there will be a constant selection for a ratio of the two types of nuclei that will permit the maximum rate of growth". On the other hand, stable submaximal growth rates were noted by BARRATT and GARNJOBST (1949) in heterokaryons of unknown nuclear proportions between wild type and colonial-1 strains, in apparent contradiction to the notion of hyphal selection.

The opportunity to test this notion is now afforded by the development of methods for experimentally controlling the initial nuclear ratio. In mixed conidial inocula, heterokaryosis is not simply the result of a few pairwise fusions of conidia, but is a widespread anastomotic process by which large numbers of nuclei are intermingled (ATWOOD and PITTENGER 1955). Therefore, it is not surprising that a systematic relation exists between the nuclear ratio in the homokaryotic inoculum and the resultant heterokaryon over a wide range of ratios (PITTENGER *et al.* 1955). Preliminary experiments proved the feasibility of constructing heterokaryons with prolonged submaximal growth rates by the use of inocula with extreme input ratios, and, in several cases, no selection could be demonstrated after various intervals of growth (PITTENGER and ATWOOD 1954). These and other experiments, reported here in more detail, confirm the absence of selection for nuclear ratios capable of giving

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the normal growth rates. A quantitative estimate of the dominance of *pan*$^+$ is given, based on the relation between nuclear proportions and growth rate.

MATERIALS AND METHODS

The homokaryotic stocks used were *pan, al-1*, A(5531, 4637); *nic-2, al-2*, A(4540, 15300); *lys-3*, A(4545), and *arg-6*, A(29997). The mutant loci are listed in the descriptive index of BARRATT *et al.* 1954. Their genetic backgrounds are such that all combinations with uncontrolled inocula give the immediate wild type response, that is, type I in the classification of HOLLOWAY (1955). Only one stock of each was used, so that the genetic background associated with a given marker is the same in all experiments. Stocks were grown at 29°C in 125 ml Ehrlenmeyer flasks, on synthetic medium supplemented with the appropriate growth factors. The preparation of heterokaryons having different nuclear ratios was as follows: (1) Conidial suspensions from homokaryotic cultures six to ten days old were mixed in various proportions; (2) mixtures were soaked overnight at 3°C in supplemented liquid medium, then incubated 2 to 4 hours at 29°C in the same medium; (3) mixtures were centrifuged; (4) the pellets were incubated 3 days at 29°C on supplemented sorbose agar; and (5) the coalesced pellets were transferred to growth tubes. It is important to note that all steps in the preparation of these heterokaryons were carried out on supplemented medium and that supplements are carried over in the final growth tube inoculum so that heterokaryosis, although established, does not become obligatory until the mycelium has grown a short distance from its starting point.

Growth rates were measured in horizontal growth tubes as described by RYAN *et al.* (1943). In some instances the growth rate did not become constant, and an average rate was determined in the region of the tube distal to that in which strong systematic trends were apparent. The growth tubes ordinarily used were 16 mm in diameter and 300 to 400 mm in length. In some experiments special growth tubes were used 1000 mm in length and 20 mm in diameter with sampling chimneys at 10 cm intervals. When growth was completed all the conidia were removed from the ends of the growth tubes or from sampling chimneys by aspiration into small glass tubes containing a tuft of glass wool. The aspirator tubes were flushed with sterile water to suspend the conidia for plating, or in some cases, the conidia were aspirated directly into a trap of sterile water. The synthetic medium of WESTERGAARD and MITCHELL (1947) was used in some experiments and Fries' #3 minimal with 3 percent washed agar (RYAN *et al.* 1943) in others. The maximum growth rate on Westergaard's medium with unwashed agar is considerably lower and somewhat more variable than on Fries' minimal with washed agar. Growth rates were measured at 29°C.

The proportions of heterokaryotic and of the 2 types of homokaryotic conidia were determined by plating conidia in a thin top layer of agar on minimal and supplemented sorbose agar. At least 3 plates of each medium were used, at dilutions yielding about 200 to 300 colonies per plate. The average number of nuclei per conidium was determined from stained preparations according to HUEBSCHMAN (1952), about 500 conidia being scored for each determination. The nuclear ratios were estimated from these data by the approximation proposed by ATWOOD and

MUKAI (1955). In some cases estimates of average nuclear number per conidium for one heterokaryon were used for others having the same components and grown under similar conditions. The variation in average nuclear number was small regardless of the strains involved, and the estimated nuclear ratio is not a strong function of nuclear number within the range encountered.

NUCLEAR PROPORTIONS WITH MAXIMAL GROWTH RATES

Nuclear proportions at the beginning and end of growth were first studied in heterokaryons having normal growth rates, but differing in initial nuclear proportions, in order to test the stability of these proportions during normal growth of a variety of heterokaryotic combinations. In one such experiment four strains, *pan,al-1*; *lys-3*; *nic-2,al-2*; and *arg-6*, were mixed in combinations of 2 in various conidial ratios not exceeding 50:1. The growth rates of the resultant heterokaryons averaged 3.1 mm/hr on Westergaard's medium and none were definitely submaximal. The data in table 1a show that regardless of the initial nuclear ratios or the component strains of the heterokaryons, the differences between nuclear proportions at the beginning and end of growth were small; none were significant when tested by the method of paired comparisons and the *t* test. The results of a similar experiment with Fries' minimal medium are given in table 1b. The heterokaryons involved the same four mutant strains as in the previous experiment, but *pan,al-1* furnished the majority of nuclei in all but one case. The mean difference between the proportion of *pan,al-1* at the beginning and end of growth was only +.016, although eight of the ten heterokaryons showed a slight increase in *pan,al-1* nuclei during growth. If this increase is real, as is suggested by the *t* test, it is clearly nonadaptive since the nuclear proportions are approaching the range in which growth rate is limited. Both experiments show that the nuclear ratio changes very little during normal heterokaryotic growth, although the growth rate is maximal over a wide range of nuclear ratios.

NUCLEAR PROPORTIONS WITH SUBMAXIMAL GROWTH RATES

Inocula with conidial ratios ranging from 50:1 to 500:1 will frequently, though not always, produce heterokaryons with reduced growth rates. The nuclear proportions and the growth rates of 21 such heterokaryons are given in table 2. Although the shapes of most of the growth curves of heterokaryons having submaximal growth rates were similar, some variation was found. The curves presented in figure 1 are representative samples of those encountered in the experiments. The shape of growth curve 12c is typical, 14 of the 21 cultures showing this particular pattern while 6 others were similar to either 13c or 14c. Inspection of the growth curves reveals that growth was initiated with a somewhat reduced lag and a relatively high rate. Subsequently, the rates of individual cultures varied for periods up to 100 hours, the early variation following a fairly constant pattern which can reasonably be related to the experimental conditions. Preincubation of the inoculum accounts for the diminution of lag, and growth factor carried with the inoculum accounts for the high initial rate. Exhaustion or outdistancing of the growth factor supply then results in a temporary cessation or retardation of growth. Finally, growth is resumed at a sub-

TABLE 1

Growth rates at 29°C and proportions of nuclear types in 20 heterokaryons sampled at the beginning and the end of 300 mm growth tubes

Heterokaryon no.	Nuclear types	Proportions of nuclear types in opposite ends of growth tubes			Growth rate mm/hr
		Beginning	End	Difference	
*a. Grown on Westergaard's medium, unwashed agar**					
30	*pan,al-1*	0.974	0.968	+0.006	3.1
	arg-6	0.026	0.032		
18	*pan,al-1*	0.920	0.946	−0.026	3.3
	lys-3	0.080	0.054		
26	*arg-6*	0.739	0.769	−0.030	2.8
	pan,al-1	0.261	0.231		
21	*arg-6*	0.696	0.747	−.051	3.0
	nic-2,al-2	0.304	0.253		
6	*arg-6*	0.749	0.729	+.020	3.3
	lys-3	0.251	0.271		
2	*lys-3*	0.796	0.813	−.017	3.3
	arg-6	0.204	0.187		
9	*lys-3*	0.785	0.834	−.049	3.2
	nic-2,al-2	0.215	0.166		
14	*lys-3*	0.802	0.780	+.022	2.9
	pan,al-1	0.198	0.220		
24	*nic-2,al-2*	0.948	0.937	+.011	3.2
	arg-6	0.052	0.063		
12	*nic-2,al-2*	0.906	0.882	+.024	3.2
	lys-3	0.094	0.118		
b. Grown on Fries' minimal medium, washed agar†					
P/N #1	*pan,al-1*	0.680	0.708	−.028	4.9
	nic-2,al-2	0.320	0.292		
7c	*pan,al-1*	0.899	0.944	−.045	5.0
	nic-2,al-2	0.101	0.056		
8c	*pan,al-1*	0.847	0.872	−.025	5.0
	nic-2,al-2	0.153	0.128		
5c	*pan,al-1*	0.961	0.976	−.015	4.9
	nic-2,al-2	0.039	0.024		
15c	*pan,al-1*	0.951	0.954	−.003	4.9
	arg-6	0.049	0.046		
P/L #2	*pan,al-1*	0.341	0.412	−.071	4.8
	lys-3	0.659	0.588		
17c	*pan,al-1*	0.859	0.874	−.015	4.9
	lys-3	0.141	0.126		
21c	*pan,al-1*	0.950	0.963	−.013	4.8
	lys-3	0.050	0.037		
23c	*pan,al-1*	0.852	0.824	+.028	4.8
	lys-3	0.148	0.176		
24	*pan,al-1*	0.769	0.745	+.024	5.0
	lys-3	0.231	0.255		

* $\overline{X}_d = -.009$.
† $\overline{X}_d = -.016$.

TABLE 2

Growth rates at 29°C and the proportions of nuclear types in 21 heterocaryons having submaximal growth rates sampled at the beginning and the end of 300 mm growth tubes

Heterokaryon no.	Nuclear types	Proportion of nuclear types in opposite ends of growth tubes			Growth rate mm/hr*
		Beginning	End	Difference	
a. Grown on Westergaard's medium, unwashed agar†					
44A	*pan,al-1*	.994	.984	+.010	1.0
	nic-2,al-2	.006	.016		
44B	*pan,al-1*	.992	.984	+.008	0.9
	nic-2,al-2	.008	.016		
55A	*pan,al-1*	.977	.940	+.037	2.0
	lys-3	.023	.060		
55B	*pan,al-1*	.974	.945	+.029	1.9
	lys-3	.026	.055		
66A	*pan,al-1*	.992	.967	+.025	2.6
	arg-6	.008	.033		
66B	*pan,al-1*	.989	.953	+.036	2.1
	arg-6	.011	.047		
77A	*lys-3*	.941	.912	+.029	2.4
	pan,al-1	.059	.088		
77B	*lys-3*	.960	.905	+.055	2.6
	pan,al-1	.040	.095		
88A	*lys-3*	.922	.945	−.023	2.2
	nic-2,al-2	.078	.055		
88B	*lys-3*	.964	.934	+.030	2.1
	nic-2,al-2	.036	.066		
99A	*lys-3*	.919	.905	+.014	2.3
	arg-6	.081	.095		
b. Grown on Fries' minimal medium, washed agar‡					
2c	*pan,al-1*	.981	.972	+.009	0.97
	nic-2,al-2	.019	.028		
3c	*pan,al-1*	.985	.982	+.003	0.98
	nic-2,al-2	.015	.018		
4c	*pan,al-1*	.982	.973	+.009	0.97
	nic-2,al-2	.018	.027		
11c	*pan,al-1*	.988	.970	+.018	1.23
	arg-6	.012	.030		
12c	*pan,al-1*	.983	.975	+.008	1.26
	arg-6	.017	.025		
13c	*pan,al-1*	.979	.964	+.015	1.16
	arg-6	.021	.036		
14c	*pan,al-1*	.963	.974	−.011	3.36
	arg-6	.037	.026		
18c	*pan,al-1*	.967	.939	+.028	1.87
	lys-3	.033	.061		
19c	*pan,al-1*	.961	.914	+.047	4.42
	lys-3	.039	.086		
20c	*pan,al-1*	.983	.950	+.033	1.44
	lys-3	.017	.050		

* Growth rates of the controls of each heterokaryotic combination were 3.0 to 3.1 mm/hr on Westergaard's and 4.9 mm/hr on Fries' medium.

† $\overline{X}_d = +.023$.

‡ $\overline{X}_d = +.016$.

maximal rate with some irregularity. Several considerations which will be mentioned in this and succeeding sections suggest that the final resumption of growth is not caused by a change in the nuclear proportions, but by some other adaptive process.

The differences between the nuclear proportions at the beginning and end of these cultures are of no greater magnitude than those in cultures with maximal growth rates. However, in all but two cases the direction of change is toward an increase of the minority type; the differences are significant by *t* test. These differences, in general, show no obvious correlation with the corresponding average growth rates, although the lowest growth rates tend to be associated with the smallest changes in nuclear proportions. This creates some difficulty for the hypothesis that the increase of the minority type is brought about continuously by natural selection, since one would expect strong selection pressure at the slowest growth rates.

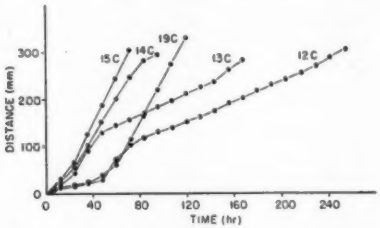

FIGURE 1.—Representative growth curves of heterokaryotic cultures illustrating the variation in shape and growth rate encountered among individual heterokaryons having submaximal growth rates. All cultures, including the control (15c) were (*pan,al-1* + *arg-6*) except 19c which was (*pan,al-1* + *lys-3*). See tables 1b and 2b for the nuclear proportions of the respective cultures.

SUCCESSIVE SAMPLING OF INDIVIDUAL CULTURES

Additional experiments were carried out in growth tubes so designed that nuclear ratios could be determined at frequent intervals during a long growth period. The purpose of these experiments was to determine whether irregularities in growth rates are correlated with changes in nuclear ratio which would not be detected by sampling the ends of ordinary growth tubes, and also whether nuclear selection would become apparent after a longer growth period. The special tubes were 1000 mm in length, with sampling chimneys every 10 cm along the top of the tube.

Six heterokaryons, *pan,al-1* + *arg-6*, *pan,al-1* + *nic-2,al-2*, *lys-3* + *pan,al-1*, *nic-2,al-2* + *lys-3*, *pan,al-1* + *lys-3*, *and lys-3* + *nic-2,al-2*, were prepared by mixing conidia in a ratio of approximately 500:1. The first of these was grown on Fries' minimal medium. The other 5 were grown on Westergaard's medium, and just prior to inoculation of each of these five, a few drops of a solution containing the growth factors of the mutants of the respective heterokaryons, were placed on the agar in the beginning of the growth tubes. This prolonged the initial period of unrestricted growth and made possible the determination of the growth rates and nuclear ratios of the heterokaryons on medium in which the growth factors of the respective mutant strain were not limiting. However, since the growth rates of the heterokaryons were faster than the diffusion rate of the growth factors in the medium, the growing fron-

tier had advanced beyond the influence of the growth factor within about 10 cm of the point of inoculation. When growth was complete, conidia were removed from most of the sampling chimneys, where they had formed in abundance, and the proportion of nuclear types determined. Four of the six cultures achieved normal rates within about 20 cm, and averaged 3.2 mm/hr on Westergaard's medium. Two of the heterokaryons, *pan,al-1 + arg-6* grown on Fries' medium, and *pan,al-1 + nic-2,al-2* grown on Westergaard's, had submaximal growth rates which averaged 1.9 and 1.4 mm/hr, respectively. The growth curves are shown in figure 2 with an additional growth curve showing a maximal rate on Fries' medium as a control for the *pan,al-1 + arg-6* culture. The nuclear proportions along the lengths of the growth tubes are given in table 3.

Although the growth rates varied considerably during the first few days of growth in the cultures having submaximal rates, the nuclear ratios were remarkably constant throughout the length of the tubes and gave no indication of changing in the direction expected to increase growth rate. Cultures having normal growth rates showed somewhat more variation in nuclear ratio, but the changes were definitely nonselective and appeared as random fluctuations except near the beginning of the tubes. In all cases where growth was initiated on medium supplemented at the proximal end with a solution of the growth factors, the major nuclear type decreased,

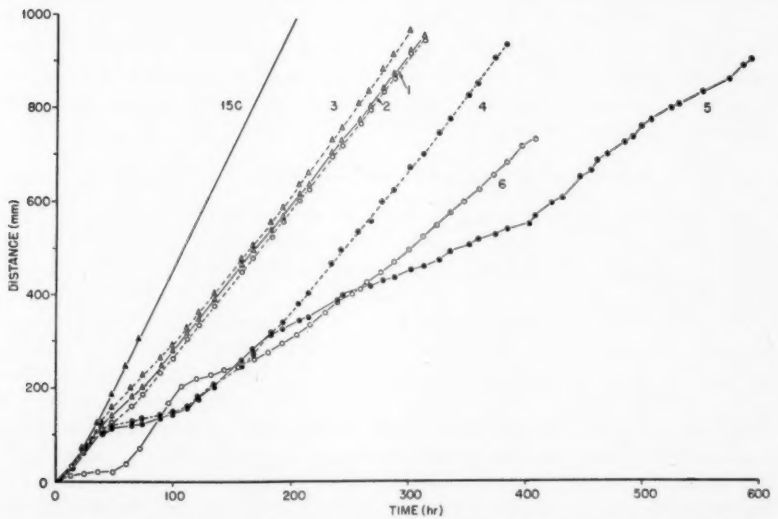

FIGURE 2.—Growth curves of 6 heterokaryons grown in 1000 mm growth tubes at 29°C, No.'s 1–5 on Westergaard's medium, No.'s 6 and 15c on Fries' medium. Growth rates and nuclear ratios for successive samples of cultures 1–6 are given in table 3. Culture 15c, grown in 300 mm tube, is included to show control rate of culture 6. The beginning of growth tubes 1–5 were supplemented with the growth requirements of the mutants just prior to inoculation. KEY: 1 = *lys-3 + pan,al-1*; 2 = *lys-3 + nic-2,al-2*; 3 = *nic-2,al-2 + lys-3*; 4 = *pan,al-1 + lys-3*; 5 = *pan,al-1 + nic-2,al-2*; 6 and 15c = *pan,al-1 + arg-6*.

TABLE 3

*Growth rates at 29°C and proportions of nuclear types in six different heterokaryons sampled at 100 mm intervals along 1000 mm growth tubes**

Heterokaryon no.	Nuclear types		Proportion of major nuclear type in the indicated sampling chimney											Average growth rate mm/hr
	Major	Minor	1	2	3	4	5	6	7	8	9	10	11	
1	*lys-3*	*pan,al-1*	.968	.936	.919		.858			.861	.924	.937	.903	3.2
2	*lys-3*	*nic-2,al-2*	.953	.926			.958	.934		.928	.962	.961	.890	3.2
3	*nic-2,al-2*	*lys-3*	.988	.939			.963			.913	.914	.914	.813	3.4
4	*pan,al-1*	*lys-3*	.970	.969	.945	.941	.945		.936		.936		.944	3.0
5	*pan,al-1*	*nic-2,al-2*	.997	.989	.990	.994	.994	.990	.986	.971	.994	.992	.985	1.4
6	*pan,al-1*	*arg-6*	.965	.976	.980	.975	.969	.966	.975	.961				1.9

* All heterokaryons were grown on Westergaard's medium except No. 6 which was grown on Fries' medium. The beginning of growth tubes Nos. 1–5 were supplemented with the growth requirements of the mutants just prior to inoculation. See figure 2 for growth curves of these heterokaryons.

although not always significantly, between the first and third sampling chimneys, or roughly coincident with the change from supplemented to minimal medium. From these results it may be inferred that consistent differences between the ends of the ordinary growth tubes in table 2 also originate near the beginning of growth, the nuclear proportions remaining fairly constant thereafter. Finally, the nuclear proportions at various sampling points in the same tube show no correlation with the growth rates locally in effect beneath these points, even in the regions where nuclear ratios and growth rates are changing most rapidly.

INFLUENCE OF EXOGENOUS GROWTH FACTORS

The presence in the medium of growth factors required by the mutant components of a heterokaryon can render the culture independent of the complementary actions of these components. Therefore it seemed possible that such growth factors would have important effects on growth rate and nuclear selection in heterokaryons. In order to observe these effects, various heterokaryotic combinations were grown on minimal and supplemented media and the growth rates and nuclear ratios were determined. In separate experiments four different heterokaryons, prepared in ratios expected to give submaximal growth rates, were grown in duplicate or triplicate on Westergaard's minimal medium and on medium singly supplemented with the growth requirements of the respective mutants. Heterokaryons within the same experiment had approximately the same nuclear ratio. The results, given in table 4, show that the cultures grown on medium supplemented with the growth factor required by the major nuclear component had normal growth rates, whereas those grown on either minimal medium or medium supplemented with the growth factor of the minor nuclear component had submaximal growth rates. The differences in the growth rates of the heterokaryons in which the *nic-2,al-2* component was predominant were not significant, but are included to show that pantothenate in the medium did not increase the growth rate, except where the nuclear proportions were already limit-

TABLE 4

The average growth rate and the proportion of nuclear types in the beginning and the end of 300 mm growth tubes of heterokaryons grown at 29°C on minimal and supplemented media in four separate experiments

Heterokaryon no.	Growth factor added to medium	Nuclear types in heterokaryon		Proportion of major nuclear types in opposite ends of growth tubes		Average growth rate mm/hr
		Major	Minor	Beginning	End	
VI-1	Minimal			.944	.941	2.3*
VI-2	Minimal			.970	.977	2.3*
VI-3	*pan*	*pan,al-1*	*arg-6*	.976	.977	3.3
VI-4	*pan*			971	.990	3.2
VI-5	*arg*			.969	.979	2.1*
VI-6	*arg*			.966	.979	2.3*
4-1	Minimal			.948	.983	2.3*
5-2	*nic*	*pan,al-1*	*nic-2,al-2*	.931	.964	1.9*
6-1	*pan*			.985	.970	3.1
PIII	Minimal			.948	.940	2.6*
PII	*lys*	*pan,al-1*	*lys-3*	.870	.866	2.3*
PI	*pan*			.939	.916	3.4
10-2	Minimal				.624	3.5
11-2	*pan*	*nic-2,al-2*	*pan,al-1*	.653	.684	3.4
12-1	*nic*			.653	.619	3.6

* Growth rate not constant during course of experiment.

ing. Changes in the nuclear ratios between the beginning and end of growth were not significant, regardless of the medium on which the cultures were grown. Thus, in heterokaryons with the stocks used, the presence of an exogenous growth factor does not confer a selective advantage on nuclei carrying the mutation for requirement of the growth factor. The absence of selection for the minor nuclear component in these experiments demonstrates again that adaptive nuclear ratio changes do not ordinarily occur whether or not growth factors are limiting.

GROWTH CURVES OF *pan,al-1* HOMOKARYONS

It seems reasonable to assume as a working hypothesis that the growth limiting effect of nuclear disproportion in heterokaryons is equivalent to a deficiency of the growth factor required by the major component. It is therefore of interest to compare the growth curves of a homokaryotic mutant under conditions of limiting growth factor supply with those of heterokaryons having the same mutant as their major component and limited by nuclear disproportion. Figure 3 shows representative growth curves of *pan,al-1* on Fries' medium supplemented with amounts of calcium pantothenate varying from 0.2 to 0.5 μg per ml. The inocula were prepared in exactly the same way as in the heterokaryon experiments except that no conidia other than *pan,al*-1 were added. It is apparent that the same sequence of changes in

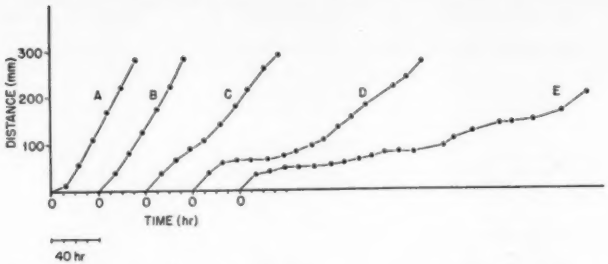

FIGURE 3.—Growth curves of *pan,al-1* homokaryons grown at 29°C on Fries' minimal supplemented with various amounts of calcium pantothenate. KEY: A = 0.50 μg/ml; B = 0.35 μg/ml; C = 0.30 μg/ml; D = 0.25 μg/ml; and E = 0.20 μg/ml. For the average growth rate of the respective cultures in triplicate, see figure 4.

growth rate occurs with the homokaryon as with various heterokaryons having *pan,al-1* as the major component. The growth rates of the homokaryon on limiting pantothenate are, if anything, less constant than those of the heterokaryons with limiting nuclear proportions. Since none of the rate fluctuations of the homokaryon can be caused by changes in nuclear proportion, it is not necessary to assume that the similar fluctuations in heterokaryons are caused by such changes.

DOMINANCE OF *pan*$^+$

Under conditions of prolonged stability of the nuclear proportions, a subnormal growth rate may be looked upon as a measure of the degree of dominance of the wild type allele present only in the nuclei of the minor component. For the purpose of correlating average growth rate with nuclear ratio and thus arriving at an estimate of dominance of *pan*$^+$, the data in tables 1b and 2b are plotted in figure 4. Cultures grown on Fries' medium afford a more reliable correlation than those on Westergaard's, because of their greater submaximal range and their less variable maximum rate. Because of the small range of nuclear proportions over which the growth rate is depressed, the frequency of the minor component is most conveniently plotted on a logarithmic scale. The frequencies of the minor component in the proximal and distal ends of the tubes were averaged, a procedure which seems justified because the differences are small in most cases, and the true frequency may often lie between these values. Actually, the results are very similar if only the distal ends are used. The average growth rates of triplicate samples of *pan,al-1* homokaryons grown on various amounts of calcium pantothenate are also plotted in figure 4 with the concentration of calcium pantothenate on the same axis as the frequency of the minor component.

The maximum growth rate of *pan,al-1* is reached with 0.5 μg of calcium pantothenate per ml and is the same as the growth rate on minimal Fries' medium of heterokaryons in which the nuclear proportions are in the optimal range. Below the optimal range, the growth rate falls precipitously with decreasing proportion of *pan*$^+$ nuclei, or with decreasing concentration of supplement. It is noteworthy that the curves are, at least roughly, of the same form; that is to say, that changing the con-

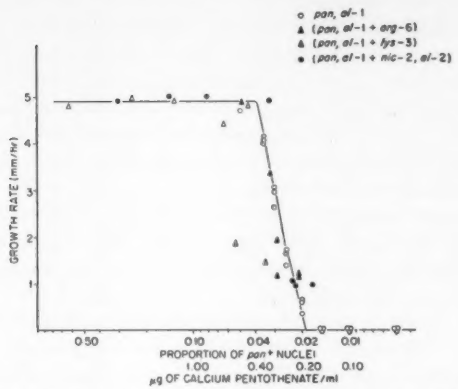

FIGURE 4.—Growth rate on Fries' minimal vs. proportion of *pan*$^+$ nuclei in heterokaryons and growth rate vs. concentration of calcium pantothenate in medium for the *pan,al-1* homokaryon. KEY:○ = *pan,al-1* homokaryon; ▲ = *pan,al-1* + *arg-6*; △ = *pan,al-1* + *lys-3*; and ● = *pan,al-1* + *nic-2,al-2*.

centration of pantothenate by a certain factor has the same effect on the growth rate as changing the proportion of *pan*$^+$ nuclei by the same factor. Thus the proportion of *pan*$^+$ nuclei can be equated to the concentration of pantothenate in the medium. From the data in figure 4 it appears that 0.1 μg of calcium pantothenate per ml is approximately equivalent in growth promoting effect to 1 percent of *pan*$^+$ nuclei.

The heterokaryons having *lys-3* as the minor component appear to require more *pan*$^+$ nuclei for an equivalent growth response than those having either *nic-2,al-2* or *arg-6*, and this effect is also apparent among the heterokaryons grown on Westergaard's medium (nos. 55A and 55B in table 2a). Although it is uncertain whether this effect is real, it is possible that this *pan*$^+$ is less dominant than the others, or that modifying factors in the genetic background of *lys-3* are responsible.

If dominance is defined as the reciprocal of the frequency of the minor component just sufficient to promote normal growth, then the dominance of *pan*$^+$ is approximately 25 to 30; that is, the maximum activity of *pan*$^+$ is some 25 to 30 times greater than that necessary for a normal growth rate. It would be of interest to learn whether the system always functions with this extra capacity, or whether the peak activity is evoked by the growth-limiting conditions. This cannot be determined with the type of information at hand, but since the growth responses to pantothenate and *pan*$^+$ nuclei are similar, it can be inferred that the activity per nucleus does not change appreciably within the suboptimal range.

DISCUSSION

During sustained growth the nuclear proportions in heterokaryons having a wide range of nuclear ratios have proved to be stable under a variety of conditions. Since the mutants used in these studies were not chosen for any special properties other than their compatible genetic backgrounds, it is safe to assume that their stability

in heterokaryons is by no means unique. The stabilizing mechanism is nonadaptive; that is, it operates to maintain the status quo even when a change in nuclear proportions would be advantageous. Certain initial shifts toward an optimal nuclear ratio, and also a rather consistent sequence of growth rate changes in the early stages of growth cannot, for the following reasons, be caused by the mechanism of selection suggested by BEADLE and COONRADT: (1) The initial trend toward increase of the minor component is not continued in a subsequent period of growth at submaximal rate; (2) during the period of initial change, the growth rate at a given point is not correlated with the nuclear proportions at that point; (3) when the *pan,al-1* homokaryon is grown on partially deficient medium the same series of rate changes is observed as in the heterokaryons.

The initial rate fluctuations are most economically ascribed to growth factor carried with the inoculum or intentionally added to the beginning of the tube. When this supply is outdistanced, an adaptation of unknown nature occurs involving a temporary lag in growth. Since this lag also occurs in the *pan,al-1* homokaryon, it is probably not related to the biosynthesis of pantothenate, but rather to processes requiring the co-factor activity. The initial shifts in nuclear proportions occurring at the boundary zone of the inoculum are probably related to the causes of dissimilarity between input and final ratios which have been discussed in a previous publication (PITTENGER *et al.* 1955). The presence of growth factor in the medium is shown to have no effect on nuclear selection during sustained growth. However, the passage of the mycelium across a boundary between supplemented and minimal medium, particularly when near the inoculum where transport mechanisms are probably poorly developed, may selectively retard the nuclei in large homokaryotic segments. The successive sampling experiments (table 3) indicate that when systematic nuclear ratio changes occur they are associated with the passage of this boundary, and later discontinued. In the indefinite period of stability which follows, it is clear that selection of fast growing hyphae does not occur.

In order to account for the stability of the nuclear ratios it is important to consider the nuclear constitution of individual hyphae at the growing frontier and the opportunity for active transport of materials from regions proximal to the frontier. As pointed out by PROUT *et al.* (1953), some of the hyphal tips isolated from the growing frontier of a heterokaryon appear to be homokaryotic. Microscopic examination of stained preparations of mycelium shows that the number of nuclei per unit length is such that it would be hardly possible, by the usual techniques, to isolate viable hyphal tips containing less than about 100 nuclei. Even if only a fraction of these nuclei contribute their progeny to a culture derived from such an isolate, the finding that such cultures are sometimes homokaryotic implies that local nuclear proportions in different hyphal tips of the same frontier must be extremely variable. At the same time, it is evident that in the heterokaryotic culture as a whole, a variation of only a few percent in the suboptimal range (fig. 4) would mean the difference between maximal growth rate and complete cessation of growth. If the growth rates of individual hyphae were dependent on the local nuclear constitution, the optimal conditions would be almost immediately restored by natural selection. However, since suboptimal nuclear proportions remain stable, it is concluded that the growth rate of

an individual hypha is not determined by its local nuclear proportions, but by the proportions within a larger domain, so that random variations are negligible. This conclusion is supported by direct examination of the growing frontier, which gives no indication of autonomous behavior of the hyphae. The configuration of the frontier changes continually during growth; sometimes it is uniform, sometimes one or more hyphae are distinctly in advance of the average position, but these hyphae do not maintain their advantage and do not replace their competitors in forming the new frontier. The growth of heterokaryotic and wild type strains is indistinguishable in this respect.

Although individual hyphae of a frontier do not exhibit permanent differences in growth rate, their behavior when isolated from the proximal mycelium is quite different. This point is illustrated by the following experiment: A *pan,al-1* + *nic-2,al-2* heterokaryon was prepared with a conidial input ratio of about 500:1 and duplicate samples of the pellet transferred to minimal agar in a growth tube and a petri plate. The growth tube culture grew at a stable rate of 1.3 mm/hr. Twenty-five hyphal tips approximately 1 mm in length were isolated from the plate culture and transferred to minimal agar growth tubes. After a prolonged lag period, eleven grew normally with a mean rate of 5 mm/hr. Eight had submaximal rates averaging 2.2 mm/hr with a range of 0.90 to 3.9 mm/hr. Six failed to grow and hence were either homokaryotic or inviable for some other reason. It may be inferred that the isolated tips possessed different nuclear ratios permitting a wide range of growth rates, or alternatively that isolation of the hyphae allowed the establishment of different nuclear proportions.

That the growth of the terminal hyphae should be influenced by the activity of more proximal regions is not surprising in view of the active transport process which has long been recognized. Microscopic examination of actively growing cultures reveals a continuous protoplasmic streaming toward the frontier of growth, the cytoplasm with its entrained particulates passing freely through the perforated septa of the mycelium. Transport of growth factors has been reported by RYAN *et al.* (1943), for distances up to 1 cm, and, under some conditions, much longer distances are involved, for instance, aerial hyphae may grow as much as 20 cm from their source of supply. A transport system extending 1 cm proximally would make available to the frontier the combined activity of enormous numbers of nuclei.

The existence of an active transport system explains the failure of hyphae to respond to local variations in nuclear ratio, but it is apparent that this is not the only prerequisite for stability. In addition, the nuclei of different components must divide at the same rate. Biosynthetic activities of the type usually affected in auxotrophic mutants have an indirect relation to nuclear division since mutant nuclei will divide normally in cytoplasm conditioned by the presence of other nuclei of complementary genotype. Limitation by nuclear disproportion may be regarded as a relative deficiency caused by the competition of a large number of nuclei for a specific biochemical activity which is facilitated by a much smaller number. This condition provides an unusual opportunity to determine whether nuclei carrying the wild type allele are as sensitive to the relative deficiency as those carrying the mutant allele. It is clear that where the suboptimal nuclear ratio is stable all the nuclei respond equally to the

biochemical deficiency whether or not they contain the allele which facilitates the synthesis of the limiting factor.

It seems certain that combinations of nuclei of some genotypes will not fulfill the condition of equal division rates, and instability of the nuclear proportions would then result. The type III heterokaryons of HOLLOWAY (1955) are examples of apparent instability; they grow normally for a limited time, then appear to have become homokaryotic at the point of cessation of growth. A nonadaptive overgrowth of *leu-1* has been reported by RYAN and LEDERBERG (1946) in heterokaryons between *leu-1* and wild type growing on limiting concentrations of leucine. Despite repeated trials, we have not succeeded in producing a heterokaryon in which growth is limited by a scarcity of *nic-2*$^+$ nuclei, or in which the proportion of *nic-2*$^+$ is sustained at less than about five percent. A possible explanation is that *nic-2*$^+$, unlike *pan*$^+$ and *lys-3*$^+$, has a selective advantage under conditions of relative deficiency. The products of the activity controlled by *nic-2*$^+$ might be more immediately available to nuclei which initiate this biosynthetic system than to other nuclei. Genetic control of the nuclear response to conditions in the cytoplasmic milieu, and of autonomously determined nuclear division rate, is to be expected; hence the mechanism of instability may be quite different in different cases, frequently nonadaptive, and frequently associated with the genetic background rather than with the markers under observation. The techniques of estimating and controlling the nuclear proportions give promise of elucidating such mechanisms.

SUMMARY

1. The nuclear proportions in heterokaryons with normal growth rates were stable between the ends of horizontal growth tubes.

2. Heterokaryons prepared from the same stocks had submaximal growth rates when extreme disproportion of the components was produced by controlling the conidial proportions in the inocula. In these heterokaryons the submaximal growth rates and extreme nuclear ratios were stable.

3. Supplementation with the growth factor, required by the major component, gave normal growth rates in heterokaryons which would otherwise have had submaximal rates, but did not alter the nuclear ratios. The growth factor of the minor component had no effect.

4. The growth responses to *pan*$^+$ nuclei and to supplementation with pantothenate were approximately of the same form. The dominance of *pan*$^+$ was estimated by correlating the nuclear proportions and growth rates of heterokaryons.

5. No evidence was found of selection of hyphae having more favorable nuclear ratios. It is suggested that this mechanism rarely, if ever, operates and that systematic changes in the nuclear ratio during sustained growth can usually be attributed to differences between the division rates of the components.

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PHENOTYPIC EFFECTS OF Y CHROMOSOME HYPERPLOIDY IN DROSOPHILA MELANOGASTER, AND THEIR RELATION TO VARIEGATION[1]

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WHEN the history of the cytogenetic knowledge of the Y chromosome of *Drosophila melanogaster* is reviewed, the impression is gained that in part at least the Y has proven so refractory to genetic analysis because, *a priori*, the cytologist and geneticist alike have always expected so little activity from it, and have tended to emphasize its relative dispensability from the chromosome set and its heterochromatic nature. Needless to say such an expectation was at one time entirely reasonable, for neither the XXY female nor X0 male (aside from infertility) show any evidence that the Y chromosome possesses a phenotypic action. But it is now known to be a cytogenetic system of very considerable complexity, although this complexity has not been worked out in even fair genetic detail. Not only does it possess a series of fertility factors (K_1 and K_2) in each arm, at least the bobbed locus which is homologous with that in the chromocentral region of X, a nucleolus organiser, some dozen or so bands when in the polytene state, and at least two specialized pairing organelles (similar to those of X, COOPER 1952), but, just as with the chromocentral regions of the other chromosomes, it has a pronounced interaction with variegating position effects.

The chromocentral regions of all of the chromosomes in *Drosophila melanogaster* are commonly held to be genetically depauperate elements, together sharing ill-defined but wide-ranging and important cytologic and genetic properties. These properties are said by most to be conferred upon these regions by heterochromatin, or more properly, are said to be owing to their heterochromatic nature. Now heterochromatin, unlike all other genetic entities, is accepted as pluripotent to an almost fabulous degree. Heterochromatin is said to affect the entire dimensions of all chromosomes of a set, to play a leading role in nucleic acid metabolism, to affect rates of development, of mutation, of chromosome pairing, of crossing over, of chromosome breakability and rejoinability, to determine localization of chiasmata, to bring about conjunction at meiosis, to affect gene penetrance and expressivity, and so on. There is very little doubt that each of these effects has been shown or made reasonable as a property within one or another heterochromatic element, but it does not seem reasonable to lump them together as attributes simply to be *expected* from heterochromatic portions of chromosomes or of entirely heterochromatic chromosomes (if such exist). Can a manifold of properties engendered by an allegedly little differentiated system, such as heterochromatin is supposed to be, be willingly accepted

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with so little proof? Not until the heterochromatic regions are genetically analysed can it be known what the actual basis for these attributes may be. At the present, however, any hypothesis which importantly involves heterochromatin is, by that very token, likely to be so elastic as to be untestable.

The Y chromosome is also heterochromatic, or heteropycnotic in the cytologist's sense, like the other members of the chromocentral set. For this reason a good number of experimenters find in it a convenient means for "adding heterochromatin" to a genotype. Regrettably there is a tendency to lose sight of the fact that a Y chromosome, not simply "heterochromatin," has been added to the genotype, and that the deductions may more properly relate to the genetic constitution of the Y chromosome than to that yet to be demonstrated catch-all, "heterochromatin." In comparison with what is known for the euchromatic lengths, there seems no question that the polytene bands of the Y are somehow related to its genic constitution, whether the bands contain genes, or simply act as spacers separating genes that carry on unrelated or mutually conflicting activities. Until more is known of the cytogenetics of the Y chromosome and of the chromocentral regions, it will probably be more profitable to endeavor to dissect their genetic systems with respect to their activities, than to add to the already impressive total of attributes assigned to "heterochromatin."

The substance of the paper which follows is partially the outcome of segregation studies in male and female complex hyperploids in *Drosophila melanogaster*, all of which were carried out at 25° ± 0.5°C. As will become clear, certain Y chromosome hyperploids and fractional hyperploids can be recognized by the visible phenotypic effects of the Y chromosomes alone. These are of a sort which brings forcibly to mind the variegating position effects, and so the problems of dosage effects by Y chromosomes and their possible relation to the V-type position effects are discussed. They are not discussed as involving attributes of heterochromatin, but as the outcome of the actions of supposed genic systems in the chromocentral elements which are subject to test and possible disproof. Whatever one's view of heterochromatin, those who consider these problems must find themselves especially indebted to the work and reflections of JACK SCHULTZ, as well as to E. B. LEWIS for his very helpful review of position effects (LEWIS 1950).

ANALYSIS OF AN APPARENTLY SPONTANEOUS MOTTLE

Among the offspring from a pair mating in a series of crosses: *Ins (1) dl-49, B*^{M1}, *sc v/ y*^{2} *apr cv v f* $\widehat{Y}^{S}$ × Canton-S (= CS) male, there occurred a single exceptional *v* female with blotched eyes, having pale washed-out areas on approximately the posterior fifths of both eyes. Bred by a CS male, the exceptional female produced 108 offspring: 11♀ and 15♂ exceptions, and 48♀ and 34♂ regular offspring. Five of the exceptional females resembled their mother, having washes of color removed from posterior portions of the eyes. In addition, two of the regular females had wild type eyes in which color was also conspicuously reduced or diluted in a more or less triangular blotch at the posterior marginal field. All other offspring seemed phenotypically normal.

These mottled-eyed F_1 females, bred by CS males, again produced some mottled

female offspring, and the irregular ratios in their progeny of mottled to non-mottled classes, as well as the absence of mottled-eyed males, suggested a genetic system involving factors of more than one chromosome. Systematic replacement of autosomes, and of the X chromosomes, in fact showed that the mottling occurred only in the presumably *y² apr cv v* $\widehat{f\,Y^s}$/ X/ Y females, for XXY daughters without the $\widehat{X\,Y^s}$ chromosome, and $\widehat{X\,Y^s}$/ X females without a Y, had unblotched eyes of normal phenotype.

Test crosses of mottled-eyed [*Mo*] *y² apr cv v* $\widehat{f\,Y^s}$/ CS/ Y females by *sc, dl-49 v, B^M1* regular sons of [*Mo*] *sc, dl-49 v, B^M1*/ *y² apr cv v* $\widehat{f\,Y^s}$/ Y females also gave numbers of mottled-eyed female offspring. Oddly, some of these [*Mo*] females proved to be *sc, dl-49 v, B^M1*/ CS in X-chromosomal constitution. It was clear, then, that the $\widehat{X\,Y^s}$-chromosome is not essential for the production of a mottled eye. Since these phenotypically wild type but mottled-eyed females gave less than 40% nondisjunction, whereas *dl-49, B^M1*/ + / Y females regularly give nearly 80% secondary nondisjunction of the X chromosomes (COOPER 1948), it was suspected that they carried two Y chromosomes. This was reasonable, for half of the fathers should have been X2Y males. In each cytologically analysed case the mottled-eyed *sc, dl-49 v, B^M1*/ CS females did in fact prove to be XX2Y, whereas their not mottled sisters were XXY. Two possibilities seemed most promising for test: (1) the vermilion alleles present in the *dl-49, B^M1* and $\widehat{X\,Y^s}$ chromosomes variegate in the presence of two Y-chromosomes, or their near equivalent (as in the $\widehat{X\,Y^s}$/ X/ Y ♀); (2) two Y chromosomes (or their equivalent) will *by themselves* cause mottling of the female eye.

The first possibility was easily eliminated. Among the regular males of [*Mo*] *sc, dl-49 v, B^M1*/ CS/ 2Y by *f B*/ Y males, CS/ 2Y males should be frequent. Crosses of such mottled-eyed females by their regular CS brothers produced some CS/ CS females which also had mottled eyes. These mottled homozygous wild type females were cytologically shown to be XX2Y; clearly, unless an unusual maternal effect was involved, or an unusual Y chromosome, the mottling must be related primarily to the number of Y chromosomes present, and not to a peculiar vermilion allele.

PROOF THAT SUPERNUMERARY Y CHROMOSOMES GENERALLY HAVE A PHENOTYPIC EFFECT

If a maternal influence is not necessary for the mottling of the eyes of a +/+, *v*/+, or *v*/*v* female carrying two Y chromosomes, nor any special genes or special Y chromosomes, then (1) it should be possible to produce mottling in a standard wild type stock simply by providing the females with two Y chromosomes; (2) on the average, half of the crosses of regular males from secondary nondisjunctional stocks by their exceptional XXY sisters should give mottled female offspring, and these mottled-eyed females should be XX2Y; (3) such mottling ought to be brought about by Y chromosomes of unrelated stock origins; (4) if the effect also occurs in males, then it is to be expected that the highly hyperploid male (X3Y or X4Y) will, like its XX2Y sister, have mottled eyes; and, finally, (5) females having a genotype equivalent to XX2Y, whether carrying free normal Y chromosomes or not, ought to have mottled eyes. Each of these expectations has been tested and, as shown below, each in fact is realized in practice. The normal Y chromosome, therefore, has a characteristic phenotypic effect when hyperploid.

TABLE 1

Crosses of XXY or attached-X/ Y females by regular sons of XXY females. These males are equally likely to be XY or X2Y. If two Y chromosomes cause the eye of a female to mottle, then one half of these crosses should produce mottled-eyed daughters

(= [Mo]♀♀) (*see text, p. 245*)

Constitution of ♀	Total no. of crosses	Number giving [*Mo*] ♀♀
CS/CS/ Y^{CS}	11	2
y v f car y v f car/ Y^{cL}	8	4
sc, dl-49 v, B^{M1}/ *y² apr v*/ Y	32	16
sc, dl-49 v, B^{M1}/ CS/ Y	17	5
sc, dl-49 v, B^{M1}/ Swed-b/ Y	27	12
dl-49 v^{o}, *f*/ *y² apr v*/ Y	33	16
Totals	128	55

$\chi^2 = 2.53$, $P_1 = 0.1$

Production of mottled-eyed females in the wild type Canton-S stock

A wild type stock of Canton-S, inbred for 120 consecutive generations by brother-sister pair-matings, was the source of the female parents. A *f B* stock, carrying the Y chromosome and all its autosomes from the Canton-S stock, provided the male parents. Pair-matings: CS♀ × *f B*/ Y^{CS} gave but 1 CS/ CS/ Y^{CS} female in approximately 12,000 female offspring.[2] By crossing the exceptional female by a *f B*/ Y^{CS} male, and continuing to do so with her exceptional female descendants thereafter, the secondary nondisjunctional stock was multiplied. Eleven pair-matings were made of CS/ CS/ Y^{CS} females by regular brothers (having an equal likelihood of being CS/ Y^{CS} or CS/ $2Y^{CS}$). Of these, two produced a number of mottled-eyed Canton-S females. Thereafter a mottled-eyed wild type Canton-S line was maintained through some 20 generations by pair-matings of mottled-eyed Canton-S females by their Canton-S brothers. The mottling of the eyes was attributable only to the presence of two Y chromosomes in the mottled-eyed females, or to some peculiarity of the Canton-S stock that results in variegation when a female carries two Y chromosomes. As will become clear, the former interpretation is the correct one.

Do half the crosses of exceptional females by regular brothers produce mottled-eyed daughters?

Table 1 summarizes the results of 128 pair-matings of regular males (equally likely to be XY or X2Y) from secondary nondisjunctional stocks by XXY or attached-X/Y females. If a female carrying two Y chromosomes generally has mottled eyes, then roughly half of these pair-matings should give mottled-eyed females among their offspring. In all, 55 of the 128 matings produced some mottled daughters; $\chi^2 = 2.53$, and $P \approx 0.1$, a result consistent with the prediction.

[2] There was also but 1 *f B*/0 exceptional male among a like number of male offspring. Such a low frequency of primary nondisjunction (0.016%) is usual when isosequential stocks are employed.

Aceto-orcein squashes were made of the ovaries of 60 of the mottled-eyed daughters from these crosses, and of 21 of their not mottled-eyed sisters. Of the mottled-eyed females (19 of which were v/v, and 41 were $v/+$ or $+/+$), 56 were in fact XX2Y. Very likely the other 4 were also, but the preparations did not reveal their karyotypes. All 21 of the not-mottled sisters were XXY (of which 17 were v/v, and 4 were $v/+$). As anticipated, then, the mottled-eyed females produced by these crosses have 2 Y chromosomes.

Mottling produced by the Swedish-b Y chromosome

It is not the case that the Canton-S Y chromosome is peculiar, or the mottling the consequence of a unique genetic quality, for the unrelated Y chromosome of the Swedish-b stock is also effective. Two secondary nondisjunctional stocks, maintained by selection, of *sc, dl-49 v, B^{M1}/ y^2 apr v/* Y* ♀♀ and X (wild type)/ Y*♂♂, provided regular females and males for pair-matings of the type: *sc, dl-49 v, B^{M1}/* X*/ Y* ♀ × *sc, dl-49 v, B^{M1}/* 2Y* ♂. In the one case the stock had the wild type X* and Y* of Swedish-b origin, in the other these chromosomes were from Canton-S. In both cases the autosomes were isosequential and all derived from the Canton-S stock. The matings were made in all combinations, and the relevant data are summarized in table 2.

Each of the crosses produced mottled-eyed females regardless of the chromosomal background, or of the origin of the X or Y chromosome. Mottling by supernumerary Y chromosomes, then, is not a peculiarity of the Canton-S Y chromosome.

Mottling in the X3Y male

Although mottled-eyed females are usual in crosses of XXY females by X2Y males, their frequency bearing an inverse relation to the rate of secondary nondisjunction, only relatively rarely do mottled-eyed males occur (e.g., from 1 to 3% of the males from ♀ *dl-49, B^{M1}/* X/ Y × ♂ X/ 2Y.) In progenies from such crosses X2Y males must be the preponderant class of males, XY next most frequent, and X3Y by far fewest of all; X4Y males, on the other hand, should not normally occur in this cross. Both by their low frequency and the fact that two extra Y chromosomes mottle the female's eye, it seemed very likely that the occasional mottled-eyed males are X3Y in constitution. This is indeed the case.

TABLE 2

Crosses of the type: sc, dl-49 v, B^{M1}/ *X*/ Y** ♀ × *X*/ 2Y** ♂. *Percent of secondary nondisjunction (XX-Y), total female offspring* (N♀♀), *and the number* (n) *and percent of mottled* [Mo] *daughters produced*

Cross and origin of X* and Y*		Percent (XX-Y)	*N* ♀♀	[*Mo*] daughters	
n ♀	in ♂			*n*	Percent
Swed-b ×	Swed-b	79.5	388	87	22.4
CS ×	Swed-b	76.9	144	29	20.1
CS ×	CS	83.1	159	27	17.0
Swed-b ×	CS	82.3	293	36	12.3

Six males having a wild type X chromosome (CS or Swed-b) and blotched eyes, and 12 males with a *sc*, *dl-49 v*, B^{M1} X chromosome and mottled vermilion eyes, all from matings of *sc*, *dl-49 v*, B^{M1}/ X/ Y ♀♀ × X/ 2Y ♂♂, were cytologically proven to be X3Y. An additional male with very slightly variegated eyes proved to be X2Y. In a second, similar series of 57 mottled males, *both* parents of which carried two Y chromosomes, 55 were X3Y and two, with only slightly variegated eyes, were X2Y (COOPER 1949). The not mottled-eyed brothers were almost all X2Y, few being XY. In no case was a not-mottled X3Y male encountered. As a general rule, then, the addition of two Y chromosomes to an otherwise normal genotype may produce a mottling of the eyes whether or not the fly is female (XX2Y) or male (X3Y).

Mottling in genotypes equivalent to XX2Y

Finally, it can readily be shown that mottling may be produced in a female whose constitution is roughly equivalent to XX2Y; for example, in a $Y^sX^{\frown}Y^L$/ $Y^sX^{\frown}Y^L$ female. "$Y^sX^{\frown}Y^L$" symbolizes a remarkable X chromosome that carries portions of both arms of Y, including all of the fertility factors, as well as all of the essential genetic material of a nearly totally inverted X chromosome (LINDSLEY and NOVITSKI 1950). Four pair-matings of: Muller-5/ *v cv* $\widehat{y\,y^+}$ (= $Y^sX^{\frown}Y^L$) ♀♀ × *v cv* $\widehat{y\,y^+}$/ 0 ♂♂ gave a total of 279 *v cv* females (*viz.*, $Y^sX^{\frown}Y^L$ homozygotes), of which 237 (85%) had mottled-eyes of varying degrees of variegation.

All five lines of evidence, then, confirm the view that two supernumerary normal Y chromosomes can cause the eye of an XX2Y female, or of an X3Y male, to become blotched or mottled. In itself this is a very surprising matter, not merely because the Y chromosome seems genetically to possess few phenotypic effects of its own, but especially because of the rather general interaction of supernumerary Y chromosomes with variegating rearrangements. As is well known, extra Y chromosomes tend to suppress the mottling brought about by rearrangements, and to restore the phenotype to normal. Thus, for example, in each case the mottling of the eyes that is induced by $T(1;3)w^{m1}$ and $In(1)w^{m4}$ is suppressed by extra Y chromosomes (GOWEN and GAY 1934; SCHULTZ 1936). It is an odd fact, then, that although extra Y chromosomes may suppress a variegation brought about by structural rearrangements, supernumerary Y chromosomes themselves can produce a variegation of the eye in the absence of such rearrangements.

THE SOMATIC EFFECTS OF SUPERNUMERARY Y CHROMOSOMES

Most conspicuous among the phenotypic effects of supernumerary Y chromosomes, in strains not carrying variegating rearrangements, are those affecting the uniformity of the coloration of the eye, but there are also effects of hyperploid normal Y chromosomes on overall morphology, and on fertility, as well. Both male and female fly seem well adjusted to the presence of a single extra Y chromosome (BRIDGES 1916), and, unless carrying variegating rearrangements, they are ordinarily of expected phenotype. No mottled-eyed XXY females have been detected by me, and only rarely has a mottled-eyed male proven X2Y rather than X3Y. Such exceptional mottled males are quite possibly mosaics of X3Y and X<3Y tissues.

Ordinarily the mottling of the eye is not sufficiently striking to draw the attention of one not examining the eyes minutely, nor is it generally easy to detect. A moderate or strong overall coloration of the eye, as well as approximately 30× magnification is necessary for the accurate classification of mottling. The following genetic backgrounds give phenotypes that, with practice, generally permit good recognition of mottling in XX2Y females: +/+, *apr*/+, *v*/+, *car*/+, *apr*/w^e, *apr*/*v*, *v*/*car*, *apr*/*apr*, *v*/*v*, *car*/*car*, *bw*/*bw*, etc. In the X3Y male, +, *apr*, *v*, *car*, *bw*/*bw* are all favorable phenotypes. Very pale-colored eyes, such as hemi- or homozygous *apr*, w^e, etc., can at times be classified reasonably well, but are not ordinarily reliable.

The *apr v*/*apr*$^+$*v* constitution of the first female fly in which Y-mottling was discovered provides an especially favorable background for its detection. The eye color of a young strongly mottled fly of this makeup may have an overall orange cast, and in ordinary illumination the mottling blotches appear as lemon yellow washes of paler color at the posterior margin of each eye. At times, and perhaps typically, the blotch in each eye is roughly triangular, with the broad base of the triangle along the posterior margin of the eye, the apex near the eye's center. At other times the wash of color may be in the form of a narrow, light streak from the eye's center to the mid posterior margin. The two eyes are generally not symmetrically affected, although they do tend to be mottled to similar extents. Only infrequently does the mottling extend to the anterior margin of the eye. When it does so, most of the posterior half of the eye is generally washed of color. Mottling, if slight, may be restricted to one eye only. It may be reduced to a patchy wash of color near the midpoint of the posterior rim of the eye, or involve only a few ommatidia, sometimes but one. As the fly ages, darkening of the eye may emphasize the color contrast of the washed-out area and normal field of the eye. For some genotypes, however, ease of detection decreases with advancing age. This is especially so in the case of wild type eyes in which the mottling grade is a mere freckling of the posterior field of the eye.

If Y-mottling is extreme, there is no problem in detecting it with ordinary illumination at 30× magnification, even in homozygous wild type flies. Commonly, and especially with weaker grades of mottling, however, the variegation of the eye is difficult to recognize unless the eye is obliquely shadowed across its surface, but otherwise brightly illuminated from the sides and below, as though in dark field (COOPER 1949). In practice this can be done conveniently by casting, from a few millimeters' distance, the shadow of the tip of a "pusher" or forceps obliquely across the eye. If, in addition, a very bright light source is used, and the flies are examined on a white porcelain tile, there is ordinarily no difficulty in observing whatever mottling may be present.

Three sorts of mottling are recognized in shadowed eyes: (1) a pale blotch or wash of color that contrasts with the overall eye-field; (2) a darker freckling of the eye (especially of the wild type eye), which may be extensive, or restricted to an ommatidium; and, rarely (3) a bright colorless point where an ommatidium is evidently devoid of pigment. Whether the seemingly darker freckles possess more pigment, or less (like the colorless ommatidia), is not known. For all types of this mottling by Y chromosomes, however, one rule concerning the region of the eye that is mottled seems to hold. Mottling commonly involves the mid-portion of the eye nearest the

posterior rim; i.e., near the 3 o'clock position for the left eye, the 9 o'clock position for the right eye. If slight, it is generally restricted to this site, and only one eye may be affected. Increasingly strong mottling extends anteriorly from this region and along the rim of the eye, widening the field of involvement. With the stronger grades of mottling both eyes are generally involved. In some stocks, furthermore, a seam of irregular facets may also be present in the dorsocaudal portion of the eye of some mottled flies.

Certain subsidiary somatic effects of hyperploidy for Y chromosomes are often present in XX2Y females and X3Y males. Where mottling can be detected, these somatic effects are generally correlated with the more extreme grades of mottling. The posterior and middle legs (infrequently the anterior legs), of one side or both, symmetrically or not, may be markedly shortened, thickened, bowed, laterally flattened and deformed, with tarsi that are twisted and curled inwards. The end of the tibia may be spatulate and coarsely bristled in an unusual manner. In very crippled flies the membrane of the wing is frequently coarse and less transparent, and in females the appearance of the abdomen may resemble that of a super-female. Such females are weakened and often of reduced viability and fertility, or even sterile. It is not known whether this reduction of fertility in females is brought about by a primary effect of Y chromosomes on the ovaries, or is a secondary consequence of the generally low viability and weakness of these extremely affected females. Whether or not the "crippling" is a primary effect of supernumerary Y chromosomes is also not known; if it is a direct interaction with certain necessary genes or factors elsewhere in the genome, these elements must be very commonly distributed through laboratory stocks in much the fashion as the apparently ubiquitous podoptera factors (GOLDSCHMIDT, HANNAH, and PITERNICK 1951).

Although colorless eyes, or nearly colorless, such as those of *w*, *apr v*, *apr v car*, etc., are not generally classifiable as to mottling, if there are strong somatic effects of the type just described there need be no great handicap to recognition of at least some XX2Y females, and X3Y males, from crosses in which hyperploid offspring are expected. While the expression of crippling is correlated with mottling of the eye, the eye need not be visibly mottled for a fly to be of crippled phenotype. Thus crippled flies occur equally frequently among the white and wild type classes of females from crosses of *w*/ + / Y ♀♀ × *y w*/ 2Y ♂♂, even though mottling of the eyes can be recognized only in the *w*/ +/ 2Y daughters. Regrettably penetrance for these gross somatic effects of Y chromosomes seems lower than that for eye mottling, at least at 25°C, so no certain judgement can be made from phenotype alone as to the Y chromosome constitution of the pale-eyed sibs that are not crippled.

THE EFFECT OF EXCESS Y CHROMOSOMES ON FERTILITY

It is a familiar fact that males which are X0 (BRIDGES 1916; SAFIR 1920), X Y^S/0, or $\widehat{XY^L}$/0 (STERN 1929b) are sterile, even though otherwise they are phenotypically normal. As STERN showed, the sterility of such males is brought about by deficiencies for fertility factors present in the Y chromosome, a set from each arm of Y being necessary for male fertility. As $\widehat{XY^S}$/Y, $\widehat{XY^L}$/Y and X2Y males are of normal fertility, or very nearly so, the conclusion seemed reasonable that overdoses of Y

TABLE 3

Fertility tests of hyperploid males having mottled eyes, hence presumably X3Y in constitution. The figures in brackets indicate the number of males in each group that was successfully analysed to chromosome constitution. Except for 3 of the 4 fertile sc, dl-49 v, B^{M1} *mottled males, all males analysed were X3Y as expected. The 44 males of established constitution are included in the total sample of 76 cytologically analysed males discussed on page 250*

Source of ♂ X-chromosome	Number of ♂♂ mated	Number fertile	95% confidence limits
Canton-S	165 [20]	1 [1]	0.06– 3.4%
sc, dl-49 v, B^{M1}	41 [17]	5 [4]*	3.9 –28.5%
car	17 [0]	0	
Swedish-b	12 [2]	0	

* 1 ♂ was X3Y, 3 ♂♂ were X2Y.

chromosomes would not have a sterilizing effect. Nevertheless, as surprising as it may seem, three Y chromosomes are sufficient to sterilize a male, as SCHULTZ first found (MORGAN, BRIDGES and SCHULTZ 1934).

The sterility of males having three Y chromosomes is demonstrated in table 3. All of the males tested for fertility had mottled eyes which were ascribable only to the effects of Y hyperploidy. Each male was mated with successive females for a period of not less than seven days, and in many cases extending to three or more weeks. An attempt was made to determine the cytological constitution of more than half of the males tested, but regrettably in only a fraction of them were division figures found and a determination possible. It is clear that the series of *sc, dl-49, v,* B^{M1} males is out of line with the others. Not only was 12% of this group fertile, but all three of the variegated males that proved to be cytologically X2Y (p. 247) possessed the *sc, dl-49 v,* B^{M1} X chromosome. Excluding this discrepant series, then, only one fertile male occurred among the remaining 195 tested males. The 95% confidence interval for fertility among X3Y males is, therefore, approximately from 0.05% to 2.9%.

The sterility of X3Y males resembles in many respects that of X0 males and other males which are sterile owing to the absence of one or more fertility factors of the Y chromosome (SAFIR 1920; SHEN 1932; COOPER 1949). The testes and allied genital structures of X3Y males are all normal in gross appearance, spermatogenesis is regular, and normal appearing bundles of spermatozoa are produced. The spermatozoa do not become motile, however, and customarily the bundles degenerate at the proximal end of the testis. Only occasionally do the paragonia and testicular ducts of the X3Y male contain free spermatozoa, but even in these instances the sperm have always proven immotile. On dissection, females that have copulated with X3Y males prove to have empty ventral receptacles and spermathecae. No doubt the rare, slight, and evanescent fertility of an X3Y male is due to a mitotic loss of one or more Y chromosomes. Such a loss might occur in a secondary gonial cell, giving rise to a cyst of X2Y (or XY) spermatocytes capable of giving motile spermatozoa. Very likely, however, the losses that confer limited fertility on these males must also involve cells destined to become nutritive cells of the testis, for motility and fertility of the spermatozoan appear to be conferred upon it by extra-spermatocytic factors, rather than by intrinsic ones (STERN 1936; STERN and HADORN 1938).

Whether or not the X4Y male is viable has not been determined. In studies of hyperploid segregations, to be reported elsewhere, a large number of crosses of XX2Y ♀♀ × X2Y ♂♂ have been made. If viable, X4Y males (from fertilizations of X2Y-eggs by 2Y-sperm) should form small fractions of the progenies. Reasoning from what has so far been found about the effects of supernumerary Y chromosomes, these hyperploids should be very strongly mottled forms (the X/X⌒Y^{S}/*sc*⌒Y^{L}/Y female is an extreme mottle), and the male at least would be expected to be sterile. Up to 4% of the mottled males from such crosses may have diminutive testes, sometimes sac-like, which in gross appearance seem empty of cells. Furthermore a small fraction of the extremely mottled females also have atrophied ovaries and are sterile. Whether these are the otherwise unidentified X4Y males and XX3Y females is not known. According to STERN's (1929a) findings, at least the XX3Y female is a viable type. Although it is an attractive thought that three Y chromosomes may also sterilize a female, it is entirely possible that these extremely mottled flies, bearing gross somatic effects as they do, are sterile not because Y chromosomes directly affect the female reproductive system, but wholly owing to their general phenotypic debility.

STURTEVANT (1945) discovered the remarkable recessive transformer gene (*tra*; 3: 44.0–45.3) which he demonstrated to convert genetic females into extreme female intersexes which are wholly male in morphology and behavior, but sterile. The sexually "transformed" flies, however, remain female in their developmental rate and in their expression of the sexually dimorphic mutants scute-3 (lethal in female), eosin, and facet. The *tra/tra* intersexes have normal male external genitalia, and a complete set of internal male reproductive organs. The testes, however, are undersized, and the addition of a single Y chromosome is apparently without significant effect for the intersexes remain sterile, and the testes small. It seemed of interest to discover whether the transformed "male" would mottle with two Y chromosomes, as its female genotype should dictate, or, like a true male, would require three Y chromosomes for mottling of the eye.

In crosses of mottled [*Mo*] X⌒X/ 2Y, *tra*/ + ♀♀ × X/ Y, *tra*/ + ♂♂, the total transformed X⌒X "♂♂" should constitute approximately one eighth of the whole progeny. If two Y chromosomes are sufficient to mottle the intersexual form, then mottled transformed "males" should be numerous and the ratio of [*Mo*] X⌒X ♀♀ to transformed [*Mo*] X⌒X "♂♂" close to 3:1. These expectations are borne out, as the following cross shows.[3]

♀♀ [*Mo*] *y* v⌒*y* v/2Y, *tra*/+ × ♂♂ *y*^{2} *apr* v/Y, *tra*/+

351 ♀ [*Mo*] *y* v, 320 ♀ *y* v, 108 "♂" [*Mo*] *y* v,

142 "♂" *y* v, and 916 ♂ *y*^{2} *apr* v; total offspring 1837.

The extra Y chromosome did not affect the sterility of the female intersexes, nor were the internal male genitalia normal in size. The testes of the four mottled "males"

[3] Regrettably two of the male parents in the 7 pair matings may have been X2Y. However, this does not affect the conclusions drawn. If *three* Y chromosomes were necessary to mottle the intersex, rather than two, and even if *all* the male parents were X2Y, at most no more than one sixth of the transformed "males" would have mottled eyes, and mottled females should be many times as frequent as mottled "males" (namely, in a ratio of approximately 12:1).

that were dissected had no more than half a coil, were lumpy along their lengths, and contained no spermatocytes, no spermatozoa, and no dividing cells. As STURTEVANT (1945) and GOLDSCHMIDT (1953) have emphasized, the genetic response of the transformed "male" is that of a female fly.

FRACTIONAL HYPERPLOIDS

Experiments on sex chromosome segregation that make use of the mottling and sterility brought about by Y chromosomes have provided interesting information on the penetrance of these effects in hyperploid females and males carrying fractional Y chromosomes. The results, summarized in table 4, permit the following conclusions to be drawn.

In both male and female, a hyperploidy of something less than + 2 Y chromosomes above normal for the genotype produces mottling in at least some individuals, and sterility among some males. However, the penetrance for mottling (i.e., the fraction of the genotype that is mottled) is not in a direct correspondence with that for the sterilizing action of Y chromosomes in males. Thus $\widehat{X}\,Y^S/2Y$ males are many-fold more fertile than $\widehat{X}\,Y^L/2Y$ males, although quite probably they are equally frequently mottled.

The degree of hyperploidy for sets of fertility factors of the Y chromosome (set K_1 in Y^L, set K_2 in Y^S) is certainly not the sole agent that determines male sterility and mottling of the eye in either sex. Since the $Y^S\widehat{X}\,Y^L/0$ male is fertile (LINDSLEY and NOVITSKI 1950), it must carry sufficient, and presumably complete, sets of K_1 and K_2. If the K-factors were decisive, then the $Y^S\widehat{X}\,Y^L/2Y$ male and X3Y male should correspond in both degree of mottling and sterility, since each carries $3K_1$ +

TABLE 4

The effect of varying degrees of Y chromosome hyperploidy on the fertility of males, and on the mottling of the eyes of males and females of favorable genotypes (*see p. 252*)

Constitution of male	Approximate percentage of class that is:		Constitution of female	Approximate percentage mottled
	Mottled	Fertile		
$\widehat{XY^S}/Y$	0	100	XXY	0
$\widehat{XY^L}/Y$	0	100	XXXY	0
$Y^S\widehat{XY^L}/Y$	0	100	$\widehat{XX}/Y^{st}/Y$	42†
$X/2Y$	<1	100	$X/\widehat{XY^S}/Y$	78
$\widehat{XY^L}/Y^{st}/Y$	8	*	$X/\widehat{XY^L}/Y$	‡
$\widehat{XY^S}/2Y$	76	40	$Y^S\widehat{XY^L}/Y^S\widehat{XY^L}$	85
$\widehat{XY^L}/2Y$	80	4	$XX/2Y$	100?
$Y^S\widehat{XY^L}/2Y$	68	11	$XX/\widehat{scY^L}/Y$	100?
$X/3Y$	100?	<1	$XXX/2Y$	‡
			$X/\widehat{XY^S}/\widehat{scY^L}/Y$	100

* Percentage not determined.

† Y^{st} is a fragment that includes the chromocentral region (XC) of X from XR through block-A, a nucleolus organizer (from either Y^S or XC), and the tip of Y^S with factors K_2. Data from MORGAN, STURTEVANT, and BRIDGES (1926), COOPER 1948 (= "Y^S" of figures and text), and unpublished.

‡ It has been determined only that at least some of this class have mottled eyes.

$3K_2$. Nevertheless the $Y^S\widehat{X}Y^L/2Y$ male is both less frequently mottled and more fertile than the X3Y male. This is borne out also by the proportionately lower frequency of mottling in $Y^S\widehat{X}Y^L/\ Y^S\widehat{X}Y^L$ females than in XX2Y females, where again the two genotypes correspond with respect to fertility factors, both having $2K_1$ and $2K_2$. Some material other than K_1 and K_2, then, from either the chromocentral portion (XC) of X, or from Y, has a strong action on mottling and sterility, and is evidently absent from the $Y^S\widehat{X}Y^L$ chromosome. DUBININ and HEPTNER (1935) have likewise given evidence for the existence in Y of a non-distal factor or factors, M_y, importantly affecting variegation.

In like fashion it is evident from table 4 that mottling and male sterility do not bear a direct correspondence with hyperploid frequencies of sex chromosomal spindle attachments, bobbed loci, or nucleolus organizers. Very likely, then, as others have contended, the materials that have genetic activity in the chromocentral region of X, and in the Y chromosome, are complex and of a nature not yet ascertained.

Although not included in the above table, attached-X stocks bear importantly on this question, for different stocks may respond in their own fashion to extra Y chromosomes. Some give strong and regular mottling in their $\widehat{X}X/2Y$ daughters, whereas others may give negligible frequencies and only a slight expression of mottling with two Y chromosomes. Sons that are X3Y, regardless of their $\widehat{X}X/2Y$ sisters' reaction to extra Y chromosomes, are regularly mottled and sterile in any case. Such results strongly suggest that the proximal regions of a normal X and Y, at least in part, are not alike.

As L. V. MORGAN (1938) pointed out, nearly all (if not all) attached-X stocks arose from oblique exchanges between the arms of either $\widehat{X}Y^S$ or $\widehat{X}Y^L$ chromosomes, or by some sequence of exchanges between X and Y chromosomes. As neither Y^S nor Y^L cytologically corresponds with XC in its basal portion (cf. figures in HEITZ 1933 and KAUFMANN 1933, 1934, or figures and summary in COOPER 1950), and as evidently there exists a number of potential exchange points between X and Y (LINDSLEY 1955), it follows that if the basal portions of Y may replace those of X, a whole family of $\widehat{X}X$ chromosomes can exist in which each member differs from the other in the composition of its chromocentral region. Such differences are adequate to account for the dependency on stock origins of the penetrance and expressivity of mottling in $\widehat{X}X/2Y$ females. For such reasons also, similar differences may be expected among different stocks of $\widehat{X}Y^L$ and $\widehat{X}Y^S$ chromosomes, and great care must be taken in judging the relative genetic effects of the separate arms of the Y chromosomes from experiments involving $\widehat{X}Y^{Arm}$.

ARE V-TYPE POSITION EFFECTS AND Y CHROMOSOMES COMPLEMENTARY IN ACTION?

Since the characteristic phenotypes of dominant and recessive V-type position effects which mottle the eyes are commonly suppressed or profoundly modified by supernumerary Y chromosomes (summary in LEWIS 1950), it may be wondered whether in turn they are directly and equally antagonistic to the action of the Y chromosomes. If so, it might be expected that the workings of the position effect and the supernumerary Y chromosomes could effectively complement one another, the result of their interaction being the simultaneous eradication of most, perhaps all,

of the phenotypic expression of each on the eye. It is also conceivable that the sterilizing effect of hyperploid Y chromosomes on males could be ameliorated for, as shown above, sterilization very likely involves the action of some elements that are not themselves K_1- or K_2-fertility factors. Two tests of these possibilities were made.

Mottled-*K* (KRIVSHENKO 1954) was used as a dominant V-type mottle. Mo^K is associated with an inversion in the right limb of the second autosome, one break of which lies within the chromocentral region. It is lethal when homozygous, but of good viability when heterozygous, producing a sharply classifiable mottling of the eyes in all XX females and XY males carrying the rearrangement. In XXY females and X2Y males, however, the eyes of Mo^K heterozygotes range in gross appearance from wholly wild type to faintly and sparsely freckled eyes. The normal Y chromosome, then, suppressed the phenotype of Mo^K. The eyes of XX2Y and X3Y Mo^K heterozygotes, however, are blotched or washed of color in the very manner characteristic of this degree of Y-hyperploidy. The dominant V-type position effect Mo^K, therefore, does not markedly counteract or suppress the action of the Y chromosomal elements that variegate the eye. The same is true for the recessive V-type position effect of $In(1)w^{m4}$.

SCHULTZ (1936) has shown that an extra Y chromosome strongly suppresses variegation of the eyes of w^{m4} hemi- and homozygotes, and this I can confirm. Nevertheless $w^{m4}/w^{m4}/2Y$ females and $w^{m4}/3Y$ males have mottled eyes as dictated by the hyperploid sets of Y chromosomes. As with Mo^K, increasing the number of Y chromosomes suppresses the position effect of the rearrangement, but the position effect itself does not appear to have an equivalent antagonistic action on the variegation of the eye that is brought about by the supernumerary Y chromosomes. Nor does w^{m4} make the X3Y male fertile. Sixteen $w^{m4}/3Y$ males were repeatedly mated with fresh females (of XX, XXY, and XX2Y genotypes); in every case the cross was sterile.

COMMENT

The effects of the Y chromosome which have been described above are almost certainly general for ordinary stocks of *Drosophila melanogaster*, and not due to some interaction between supernumerary Y chromosomes and a particular mutant gene or rearrangement peculiar and common only to the stocks used in these experiments. SCHULTZ (1941a, and in: MORGAN, BRIDGES and SCHULTZ 1935), for example, has already noted that the X3Y males are a sterile class. It is not possible to say for certain whether the variegating effects of supernumerary Y chromosomes have been observed before, although many have mentioned crosses or occurrences of XX2Y females, and of X3Y males. While it may be that the seemingly anomalous effects of supernumerary Y chromosomes on variegation of the light locus (SCHULTZ 1936, and in: MORGAN, BRIDGES and SCHULTZ 1934; MORGAN and SCHULTZ 1942), or on that of bw^4 (DUBININ and HEPTNER 1935), are actually instances of the Y chromosome effect on the eye that I have just described, the presence of light or any other mutant locus is not normally required. In any event, the phenotypic responses of both light and bw^4 are explicable in terms of the general hypothesis offered in the discussion below.

Although it is true that the flies in which a Y-induced variegation of the eye was first observed were actually of a very complex genetic nature (see p. 243), it has been shown that flies of pure Canton-S wild type lines variegate when suitably hyperploid for Y chromosomes, as do strains derived from Swedish-b and many other diverse stocks, presumably little related, that were obtained from the California Institute of Technology, Indiana University, the University of Missouri, Cold Spring Harbor, and elsewhere. Not only is the effect shown within stocks having autosomes and X chromosomes of different origins, but the Y chromosomes too need not be of any particular strain or sort. Y^{CS}, $Y^{Sw\text{-}b}$, $sc^{s}\cdot Y$, $Y{:}bw^{+}$, $sc\cdot Y^{L}$, and so on, are all at least very similar in this respect, for when they are members of a set of Y chromosomes in an XX2Y female, or in an X3Y male, the phenotypic effects on the eye and on male fertility are alike.

The fact that supernumerary Y chromosomes mottle wild type or self-colored eyes makes it possible to carry out some segregation studies not otherwise feasible. Nevertheless considerable care is necessary, if mottling alone is to indicate the Y chromosome constitution, for both penetrance and expressivity fluctuate within and between stocks. Although flies with variegated eyes that occur in progenies from XX2Y females by XY males, or from XXY females by X2Y males, may generally be classified with confidence as XX2Y females and X3Y males, non-mottled flies of these constitutions may also occur. Furthermore it is a surprising fact that both hyperploid males and females may scale down the Y-hyperploidy within the gonad, even though retaining a higher somatic hyperploidy. This is most convincingly observed in flies with suitable Y chromosome markers; e.g., in $y\ v\cdot = /sc^{s}\cdot Y/Y{:}bw^{+}$; *bw/bw* females and $y\ v/Y^{CS}/sc^{s}\cdot Y/Y{:}bw^{+}$; *bw/bw* males. In each instance the occurrence of a vermilion eye (i.e., not *v bw*) and a wild type body color (owing to the y^{+} allele of $sc^{s}\cdot Y$) indicates the presence of both marked Y chromosomes in the soma, and the eyes of such flies may be strongly mottled. Yet some of these males may be fertile to a normal degree, and in each such case they have bred as $y\ v/Y^{CS}/sc^{s}\cdot Y$; *bw/bw* males. In other words, $Y{:}\ bw^{+}$ has been lost in at least a large sector of one or both gonads of such males. In similar manner an occasional female transmits only one of the two Y chromosomes to her offspring. The reverse cases, in which somatically *y v* not-mottled females and males (or *v bw* females and males) have transmitted both $sc^{s}\cdot Y$ and $Y{:}bw^{+}$ to their offspring, have not been observed. Whether the seemingly high frequency of fertile *sc*, *dl-49 v*, B^{M1}/ 3Y males (see p. 250) is a consequence of such a gonadic scaling down of Y chromosomes is not known.

DISCUSSION

As earlier remarked, the mottling of self-colored eyes which occurs in the presence of supernumerary Y chromosomes is perplexing for, as is well known, Y chromosomes commonly suppress the mottling brought about by variegating (or V-type) position effects (GOWEN and GAY 1934; SCHULTZ 1936, and in: MORGAN, BRIDGES and SCHULTZ 1935, reviews in: SCHULTZ 1941a, b, 1948, 1952; LEWIS 1950; HANNAH 1951). As a general rule, striking V-type position effects in *Drosophila melanogaster* involve displacements of chromosomal elements (MULLER 1930) to and from chromo-

central regions (SCHULTZ 1936), with the formation of new euchromatic-chromocentric associations. In *D. virilis*, at least, V-type position effects may also arise in the case of purely chromocentral displacements (BAKER 1953), and this may prove to be the case in *D. melanogaster* as well (STERN and KODANI 1955). In striking contrast, wholly euchromatic rearrangements give rise preponderantly to stable, S-type position effects (LEWIS 1950). Chromocentral change, therefore, is a cardinal element in variegating position effects, although not all displacements involving chromocentral elements result in detectable variegation.

The euchromatic genes (and, in *D. virilis*, chromocentric) involved in the production of variegated phenotypes are of no particular sort, but as a group are necessarily characterized by the production of terminal, autonomous, cellular differentiations (MULLER 1930; LEWIS 1950). Both the stability of phenotype and the normal action of such genes may be restored when the variegating euchromatic locus is removed from its chromocentral involvement, in whole or in part, as in the cases of some induced reversions of w^{m11} (PANSHIN 1938), of rst^3 (KAUFMANN 1942), and presumably of variegating *In(2LR)40D* (HINTON 1950). In these cases, the position effects largely or entirely disappear. Nevertheless, the obvious fact must be kept in mind that the original change responsible for the V-type position effect may have conjointly involved position effects on chromocentric loci, as well as those on the euchromatic loci to which attention has chiefly focussed. Whatever the case may prove to be, as LEWIS (1950) so aptly comments, the common attributes of the V-type position effects suggest a division of the genetic material into two major components, one cytologically corresponding with the euchromatic lengths of the polytene chromosomes, the other with the chromocentral regions.

It is by no means a simple matter to account for variegation in terms of what is known. None of the older interpretations that require mutable loci, unstable rearrangements, excessive reduplication of affected loci, duplications undergoing somatic crossing over, abnormal reproduction of genes in twisted genonemata, or in lateral attachments, mechanical asymmetries and torsions imposed on loci by somatic pairing of heterozygous rearrangements, modes of conformation of the chromocenter more or less persisting in groups of related cells, and so on, are reconcilable with the facts as they now stand. Nor do such explanations, or conceivable ones involving the simple introduction of euchromatic elements into the chromocentral environment normally foreign to them, make understandable the suppression of mottling in otherwise variegating genotypes to which chromocentral elements, or of duplications of chromocentral elements, have been added in the form of Y chromosomes, or the occurrence of mottling in normal genotypes hyperploid for Y chromosomes.

The chemical or "kinetic" interpretations of position effects which stress interference with immediate or early gene products (OFFERMANN 1935, and others), or changes in competitive combining powers and reaction efficiencies of genes (STERN and HEIDENTHAL 1944), or accumulations of precursors (LEWIS 1950), or interactions of substrates (BAKER 1953) or of gene products (BELGOVSKY 1946), and so on, can in principle explain the occurrence of a phenotypic change, or apparent inactivation of a gene, consequent to rearrangement, but none bears an implicit relation that would automatically explain variegation of phenotype. Something in addition is required.

More than any other, SCHULTZ (1936–1952)[4] has emphasized the role of the chromocentral elements in variegation. They are viewed by him as nullifiers of euchromatic loci newly conjoined to them, and in this kind of interpretation he has been followed and supported by NOUJDIN (1938, 1939, 1946a, b), PROKOFYEVA-BELGOVSKAYA (1939, 1946, 1947), and others. Variegation is envisaged as a consequence of "heterochromatization," or effective somatic inactivation of euchromatic loci brought into close continuity with chromocentral material. The inactivation might be in a literal sense, or the displaced euchromatic loci, inefficiently competing with those of the chromocenter for nucleic acids, might fail to be reproduced in certain somatic cells and thus be lost in a sort of intrachromosomal diminution. Or again, acquiring properties inferred from the allometric growth of the chromocentral elements in nurse cells (SCHULTZ 1948, 1952; and in: MORGAN, SCHULTZ and CURRY 1941), they are viewed as retarded in their multiplication when compared with that of the normal euchromatic complement. Since "heterochromatization", a process which seems to encompass all of these effects, and still others, is variable from cell to cell, variegation of phenotype would characterize the action of rearranged euchromatic loci that have autonomous effects.

SCHULTZ explains the suppression of variegation when Y chromosomes are added to the genotype (presumably also when other chromocentral elements are added), by holding that the general pool of nucleic acids is adequately heightened by their presence. Displaced euchromatic loci thus would become capable of maintaining normal rates of reproduction and action, and would not heterochromatize, for they would no longer need to be starved by their more or less ineffective competition with adjacent chromocentral elements for nucleic acids at threshold levels. This has been a fruitful view, even though containing what seem to be contradictions and being subject to dispute regarding important facts (PROKOFYEVA-BELGOVSKAYA 1939; COLE and SUTTON 1941; CALLAN 1948; GERSCH 1949; SCHULTZ and CASPERSSON 1949). Nevertheless, even within the wide limits which such an interpretation grants, it is difficult to account for the mottling of the eye that accompanies Y chromosome hyperploidy in otherwise normal, non-variegating genotypes.

NOUJDIN (1938), DEMEREC (1940, 1941), RAFFEL and MULLER (1940), KAUFMANN (1942), LEWIS (1950), and HANNAH (1951), have all emphasized that just as with the euchromatic break, the locations of breaks in the chromocentral elements which are associated with V-type position effects are important, and that the chromocentral regions are by no means of a linearly uniform property with respect to causation of variegation. It is not merely necessary that the affected euchromatic locus be continuous with, or adjacent or subadjacent to a chromocentral element for variegation to occur, but the chromocentral break must include or neighbor a sensitive site. Such responsive sites might be imagined as more or less repeated loci of special function occurring in the chromocentral regions much as PONTECORVO (1943) has portrayed heteropycnotic regions adjacent to kinetochores. Just as position effects may "spread" along a euchromatic length (MULLER 1930; MULLER and PROKOFYEVA 1935; DEMEREC

[4] SCHULTZ has contributed most widely to the subject of variegation, and those interested in the full range of his discoveries and views should refer also to his contributions in: MORGAN, BRIDGES and SCHULTZ 1933–38; MORGAN and SCHULTZ 1942; MORGAN, SCHULTZ, BRIDGES and CURRY 1939; and MORGAN, SCHULTZ and CURRY 1940–41, and in SCHULTZ and CASPERSSON 1939; 1949.

1940, 1942), BAKER (1954) has shown that they may "spread" along chromocentral ones in *D. virilis*. It is quite possible, then, that chromocentral "spreading" may also occur, and this could account for disbalances arising from a simple rearrangement even were the numbers of relevant loci within a chromocentral element quite high.

As earlier commented by SCHULTZ (1936–1952, and in: MORGAN, BRIDGES and SCHULTZ 1934, 1935), chromocentral loci may be viewed as together forming a system which is in balance with loci elsewhere in the euchromatic lengths. As a purely formal way of looking at it, we may suppose that normal autonomous differentiations within cells require a state which is a consequence of euchromatic-chromocentric interaction, and that this state is of some range in latitude or time. Changes in the euchromatic-chromocentric balance which may occur through ordinary fluctuations in genotype (for example, by nondisjunction of sex chromosomes) would, in the long run, act selectively to affect the adjustments of those genes sensitive to the results of balance and unbalance. Were this so, thresholds of individual loci primarily concerned with autonomous cellular differentiations might differ, provided all fall within the narrowest limits of the normal range in state. Thus the actions of genes need not be impaired by displacements not including or affecting them, provided that any changes in balance which may occur do not fall below their threshold requirements. But the displaced loci, if themselves subject to position effect in much the way STURTEVANT (1925), or OFFERMANN (1935), or LEWIS (1954) conceive, might now operate outside, or marginal to, their present needs and variegation result, for then differentiation would not be uniform from cell to cell. The addition of one extra Y chromosome would suppress the V-type position effect if the state supposedly brought about by euchromatic-chromocentric balance were shifted to a point that the displaced, variegating gene no longer acted under marginal or limiting conditions. Were unbalance extreme, even genes that were not displaced might variegate, and it is assumed that some such gene or genes are responsible for the variegation in hyperploids having two Y chromosomes above normal. Just as maleness or femaleness in Drosophila is evidently determined by an unbalance of autosomal male-trending genes with X-chromosomal female-trending genes, and intersexuality by varying degrees of balance (BRIDGES 1932), in this case variegation is also interpreted as a consequence of unbalance.

A balance interpretation of variegation has much to commend it, for V-type position effects on euchromatic loci may be enhanced by heterozygosity for amorphic alleles or deficiencies at the locus (LEWIS 1950), by homozygosity for the rearrangement (DEMEREC and SLIZYNSKA 1937; SCHULTZ 1941a, etc.; LEWIS 1950), and especially by deficiencies for chromocentral elements as in the X0 male (SCHULTZ 1936; NOUJDIN 1936) and in individuals deficient for the chromocentral region of 2R (SCHULTZ 1941a, and in: MORGAN, SCHULTZ, *et al.* 1940, 1941). Conversely they are suppressed by the addition of Y chromosomes, and by duplications of chromocentral material from 2R, and from the 4th and X chromosomes (SCHULTZ 1941a, and in: MORGAN, SCHULTZ and CURRY 1940; NOUJDIN 1938, 1939, 1946a, b). Furthermore, as SCHULTZ (1941a) has found, V-type position effects are nevertheless expressed in intersexes even in the presence of an extra Y chromosome. Finally such an interpretation can encompass the production of variegation in Y-hyperploids with

otherwise normal genotypes, for in principle a point of unbalance ought to be attainable at which even some normal alleles would operate outside the threshold limit at which their full phenotypic expression is regularly reached.

It is true that I have not observed variegation other than that of eye coloration (and sometimes corneal defects) in wild type Y-hyperploids. It is also the case that V-type position effects may involve not only eye coloration and corneal irregularities, but also integumentary coloration, presence and absence of hairs, bristle form, and so on, and all seem suppressable by the simple addition of Y chromosomes. How is it then that the wild type Y-hyperploid is not conspicuously variegated for these characters? It may be that sufficiently high levels of disbalance simply are not achieved or, if achieved, their effect may involve so many elements of differentiation that it proves lethal. Certainly the frequent somatic deformation in highly hyperploid flies (see p. 249) bespeaks the possibility of more general effects than those on the eye alone.

In the same fashion, but probably involving other genes, a balance interpretation can adequately account for the sterilizing action of supernumerary Y chromosomes on males, as SCHULTZ (1941a; and in: MORGAN, BRIDGES and SCHULTZ 1934, 1935) has already noted. A male of *Drosophila melanogaster* requires the two different fertility groups of the Y chromosome for sperm motility and fertility. But these, and very likely other factors in the Y chromosome (see p. 252), may be viewed as opposing the actions of genes elsewhere in the chromosome set. When too many Y chromosomes are present, the effect of the genes of Y would exceed the balance and the male be rendered infertile. It remains to be shown what effect, if any, such disbalance may have in the female.

The cytologist is familiar with numerous instances, as in coccids (HUGHES-SCHRADER 1948), cecidomyids, and other insects (WHITE 1954), in which balances may be maintained, in at least certain tissues, between a differentiated class of chromosomes and the remainder of the set. On the whole, balanced or redundant systems have many attractive aspects for the interpretation of development and the control of phenotype, no less for the explanation of gradual yet extensive evolutionary changes in the genotype despite an overall retention of the original basic genetic system. They are furthermore very susceptible to direct genetic test, and it is precisely this aspect of the interpretation of V-type position effects just outlined which makes it seem worthwhile.

Although the unification of the effects of simple Y-hyperploidy with that of the V-type position effects is easily and naturally encompassed in this admittedly broad view, it may still prove to be the case that the variegation of wild type (or non-mottled) eyes by supernumerary Y chromosomes is an epiphenomenon, parallel to, but not immediately involved with V-type position effects. It is odd that w^{m4}/3Y and X/3Y, Mo^{K}/+ males both show the characteristic dorsocaudal blotching of the eye characteristic of variegation by Y chromosome hyperploidy alone, whereas w^{m4}/2Y and X/2Y, Mo^{K}/+ males are wild type or nearly so (p. 254). After all, *two* additional Y chromosomes are required to variegate the wild type eye, while in the above cases only *one* additional Y chromosome seems to give variegation. At first glance it would seem that in these cases the Y chromosome is carrying out two

independent actions, rather than merely recreating, and then superseding in the usual way, a balance with opposing, non-chromocentral elements. Nevertheless SCHULTZ (1943) has discovered a w^{m4} derivative that requires not one, but two Y chromosomes for suppression of its variegation. It remains to be seen whether study of loci such as this one, as well as accumulations of unrelated V-type position effects within one genotype, will provide evidence consistent with the general balance interpretation and with the view that Y-induced variegation is a consequence of upset of this balance.

Finally, it must have been noted that I have deliberately employed "chromocentral region," rather than "heterochromatin," throughout my discussion. "Heterochromatin" today is what an investigator chooses to make it or imagine it, and I agree wholeheartedly with the views on this score expressed by BAKER and CALLAN (1950). "Heterochromatin" occurs in fully 17 of the 20 numbered divisions of the polytene X chromosome (PROKOFYEVA-BELGOVSKAYA 1939; KAUFMANN 1944, 1946; SLIZYNSKI 1945), if by "heterochromatin" we mean only sites of ectopic pairing or of unusual breakability or rejoinability. "Heterochromatin" also occurs in the Y chromosome and all chromocentral regions. But these latter are differentiatable genetic systems, and it would seem best to demonstrate first the propriety and usefulness of referring their actions to allegedly common heterochromatic properties, or to different kinds of "heterochromatin," rather than directly to their genetic constitutions. Conversely, there is little logic to the transfer of the genetic properties of Y chromosomes and chromocentral regions to the "interstitial heterochromatin," of which few facts are known but to which much is imputed. Analysis of the genetic organization and action of the chromocentral elements has barely begun, and quite possibly it holds keys to many of our genetic puzzles, as, for example, position effect.

SUMMARY

1) Y chromosomes have a characteristic pair of phenotypic effects in *Drosophila melanogaster* when there are two above normal for the genotype, namely in XX2Y females and X3Y males: they cause a mottling of the self-colored eye, and they sterilize the male. These effects are shown to occur in a number of unrelated strains, and with a number of different Y chromosomes. They are probably general within the species.

2) There are frequently other somatic irregularities observable in flies hyperploid for Y chromosomes: eye facets may be irregular, the legs may be shortened and deformed, the membrane of the wing abnormal, and so on. These effects have not been studied, and may depend on interactions with certain genes that are common in laboratory strains.

3) XX2Y, *tra/tra* females that are transformed to extreme male-type intersexes variegate just as their female genotypic constitution dictates; they remain female in their response to the supernumerary Y chromosomes.

4) Fractional hyperploids demonstrate that something less than two supernumerary Y chromosomes is sufficient to cause mottling, and that the factors concerned with sterilization of hyperploid males are at least in part independent of

those causing variegation of the eye. Furthermore, sterilization of males involves the action of some elements that are not themselves members of the K_1- and K_2- fertility groups.

5) The proximal regions of the X chromosome and Y are, at least in part, not alike cytologically, or in their direct genetic contributions to variegation. In principle, a family of attached-X chromosomes must exist, the members of which differ in their chromocentral components. In this way the variable response among different attached-X stocks to Y chromosome hyperploidy may be explained.

6) The V-type position effects of Mo^K and w^{m4} are suppressed, in whole or in part, by one extra Y chromosome; nevertheless they themselves evidently do not prevent expression of the characteristic variegation brought about by two extra Y chromosomes.

7) XX2Y females and X3Y males occasionally become mosaic for the Y chromosomes so that the original constitution is retained in the soma, but a Y chromosome is lost in the germ tract. Such mosaic males are fertile.

8) V-type position effects are discussed, and a purely formal explanation involving a euchromatic-chromocentric balance is proposed, of a directly testable nature, which can account for both the suppression and production of variegation by supernumerary Y chromosomes, as well as for the V-type position effects themselves. Despite the suggestion of such an all-encompassing view, it remains possible that the variegation caused by Y chromosomes is an independent phenomenon.

9) The sterilization of 3Y males may also be explained in terms of a genotypic balance.

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SEX INSTABILITY IN RICINUS

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CHANGES in sex expression occur occasionally in individuals of different species of plants. These changes have been attributed, and are often due, to fleeting non-hereditary variations. However, studies of sex expression, during the past five years, have revealed that similar changes may be associated with hereditary instability. In this paper, a description is given of the phenotypic and breeding behavior of various sex forms in *Ricinus communis* L., the castor-oil plant, with particular reference to changes which occur during development.

GROWTH AND FLOWERING HABIT

The habit of growth and flowering of Ricinus is presented diagrammatically in figure 1A. The plant is determinate; the main stem first and later all subsequent shoots terminate in a floral cluster. New shoots can be produced from practically all nodes, but, with few exceptions, branching begins only after the primary inflorescence has appeared at the apex of the main stem. The number and length of internodes preceding the primary inflorescence determine whether a given plant is early or late, dwarf or giant. The castor-oil plant is potentially a perennial tree. A single individual of an early variety may bear over 200 inflorescences during its first year of growth and, under favorable conditions, it will continue to bloom for several years.

This monotypic genus ($2n = 20$) is primarily wind-pollinated and all known variants, including previously described species, intercross readily and produce fertile offspring. The plant is typically monoecious and the inflorescence is racemose. The raceme (fig. 2, A) is made up of a main axis and alternate compound branches which usually bear unisexual flowers; pistillate flowers are borne on the upper branches of the raceme and staminate flowers on the lower branches. The ratio between pistillate and staminate flowers in the racemes of a given plant is the index of sex tendency of that particular plant. In the common monoecious varieties the ratio between pistillate and staminate flowers is about 1:2. Races having higher or lower ratios manifest stronger female or stronger male tendencies respectively. The number of flowers per raceme varies considerably among different strains but it is usually over 120.

SEX VARIATION

Besides extreme racial differences in sex tendency, there are female variants among all castor populations. Instead of bearing both pistillate and staminate flowers, as do racemes of ordinary monoecious plants (fig. 1, A), at least some of the racemes of these female individuals bear pistillate flowers only (fig. 1, C–F). ROXBURGH (1874) discovered a female castor tree in India which led him to believe that there exist dioecious species in this genus. JOSHI (1926) and, later, other workers reported on the occurrence of wide variations in sex tendency among castor inflorescences, including strictly pistillate racemes. What is the nature of these variations?

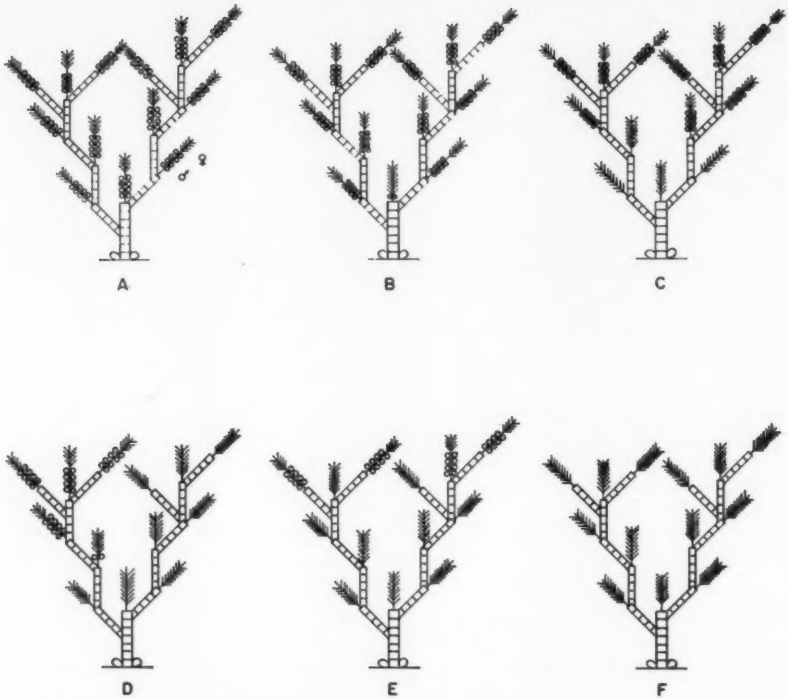

FIGURE 1.—Diagrams of some developmental patterns of sex expression in Ricinus. A, a monoecious individual; B to E, sex reversals; F, strictly female at this stage, but capable of reverting to monoecism depending on genotype.

Sex tendency in castors is subject to extreme non-genetic fluctuations. Female tendency is relatively strong in young plants, especially in primary racemes, and under conditions of moderate temperatures, moderate vegetative activity, and high level of nutrition. Furthermore, female tendency may increase most conspicuously following severe pruning of well established plants. In contrast, male tendency is relatively strong in old declining trees and under conditions of very high or fairly low temperatures, high vegetative activity, and low level of nutrition. While a combination of extremely high temperature and low level of nutrition may lead to a temporary state of complete maleness in plants of some varieties, certain cultural conditions, notably pruning, may bring about a temporary state of complete femaleness in races which ordinarily give a high pistillate-staminate ratio. Such environmentally-induced females turn quickly to monoecism and, if selfed, they invariably breed true to monoecism.

All castor races respond to seasonal variations, irrespective of the stage of development of individual plants. In Israel, femaleness is strongest in spring and early sum-

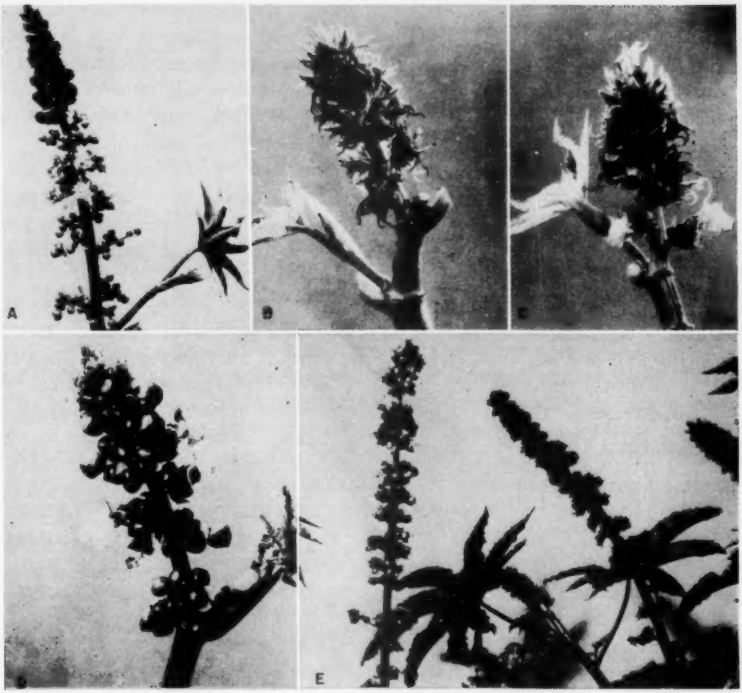

FIGURE 2.—Sex expression in different racemes of Ricinus. A, a normal raceme of the variety Herdocia; B, C, spring and winter racemes from the female inbred Nebraska; D, a raceme of the strongly male inbred 97 bearing a terminal hermaphrodite; E, female racemes from a sex-reversal plant of Queen 162; on the right a strictly pistillate raceme representing the prevailing type in spring and, on the left, a female raceme bearing interspersed staminate flowers representing the type occurring intermittently in winter.

mer, April to June. Strong male tendency is manifested intermittently during mid- and late-summer, July to September, and again during winter, January to February.

Genetic data on sex inheritance in castors were presented by KATAYAMA (1948) and CLAASSEN and HOFFMAN (1950). These workers describe a gynodioecious race in which monoecious and female plants occur in a ratio of 1:1, monoecious individuals being heterozygous. DR. C. E. CLAASSEN, formerly of the University of Nebraska, has generously furnished us with seed of such a backcross race, N145-4. Individual females of N145-4 produce intermittently a few staminate flowers at the base of some racemes during summer and later during winter (fig. 2, B and C). This behavior has enabled us to establish a female inbred known as Nebraska and to use its pollen in some crosses. Nebraska is strictly pistillate in spring and early summer in Israel.

As in a previous study of fruit color in Cucurbita (SHIFRISS 1949), a developmental approach was adopted in the present investigation of sex variation. Accordingly, the

habit of growth and flowering of every plant tested was diagrammed with particular reference to time of appearance, sex expression, and position of each individual raceme. This developmental approach, in addition to breeding records, has helped to discern between hereditary and non-hereditary variations which often occur during the life of individual plants. It was quickly discovered that, unlike Nebraska which is quite stable phenotypically except for slight intermittent seasonal changes, the more common female variants revert sometime in their life to a permanent state of normal monoecism. Furthermore, in contrast to Nebraska, these sex-reversals do not breed true. With due consideration to the various facets of the problem, all sex forms have been divided into two major categories, according to their breeding behavior; genetically stable and genetically unstable sex forms.

The genetically stable forms include pistillate and monoecious variants all of which breed true. Under a given set of conditions, the homozygous monoecious races vary in their pistillate-staminate ratio from 2:1 to 1:14 (fig. 2, D). These races also differ from each other in their relative hereditary potentiality to form a terminal hermaphroditic flower at the apex of individual racemes, as well as in their ability to produce, under a given set of conditions, staminate and/or hermaphroditic flowers in the pistillate portion of the racemes. Our studies have confirmed and extended the findings of KATAYAMA and CLAASSEN and HOFFMAN. Accordingly, alleles of a major gene *F* differentiate between the various monoecious races, as well as between these races and the female inbred Nebraska, *f*; gene *F* is independent of the genes for plant color and waxy bloom but is linked with some of the polygenes which control the number of nodes preceding the primary raceme.

The genetically unstable forms consist of sex-reversals and monoecious individuals which do not breed true. These sex-reversals, which turn from femaleness to monoecism at different times in different regions of the same plant, can be divided further into various types and stocks depending on their relative capacity to generate sex-reversals. They also vary from each other in their ability to produce, under certain circumstances, interspersed staminate flowers in some of their pistillate and normal racemes. A fuller description of the phenotypic and breeding behavior of unstable stocks will be presented later.

INCIDENCE OF NATURALLY OCCURRING SEX-REVERSALS

An extensive search has been conducted for the occurrence of spontaneous pistillate plants in different castor populations which the writer has surveyed in Israel, Morocco, Kenya, Tanganyika, the Rhodesias, and in the Union of South Africa. Over a million plants of 48 different races were scanned. All female individuals found in this search proved to be sex-reversals, i.e., pistillate plants which are capable of reverting into *normal* monoecism sometime in their life (fig. 1,C–E).

Sex-reversals were discovered in practically all spontaneously grown populations, horticultural varieties, and artificially-developed inbred lines. The frequency of their occurrence is relatively high, but it varies considerably depending, among other things, on the breeding history of the material. In general, the frequency is higher in specially selected stocks and in open pollinated populations than in recently-bred monoecious strains.

TABLE 1

Incidence of naturally occurring sex-reversals

Variety	Total plants observed	Number sex-reversals	Frequency sex-reversals
Adamdam	40,000*	26	1:1,538
Baker No. 1	28,500	5	1:5,700
Blackwell	32,720	3	1:10,907
Cimarron	43,400	8	1:5,425
Early Spineless	82,000*	5	1:16,400
H.S. 1	1,500	4	1:375
Kehalhal	16,836	15	1:1,122
Oued Beth	250,000*	141	1:1,773

* Approximate number.

Some data on the frequency of occurrence of sex-reversals are given in table 1. Of special interest is the finding of 141 sex-reversals among a total of about 250,000 plants in the open pollinated Moroccan variety Oued-Beth, a frequency of about 1:1800. The incidence of sex-reversals in some of the newly introduced American varieties such as Baker No. 1, Blackwell and Cimarron is rather low. On the other hand, certain stocks of such varieties as U.S. 72, which consist of about 25 percent sex-reversals, have undoubtedly originated through selection of sex-reversals which often appear as superior yielding plants.

It is not unlikely that some of the naturally occurring sex-reversals are environmentally induced. Whether a specific sex-reversal is genetically controlled or not can be determined by its breeding behavior. Since breeding experiments, to be described later, have shown conclusively that the majority of the naturally occurring sex-reversals are genetically unstable, it can be concluded that the hereditary instability affecting sex, and manifested by the occurrence of sex-reversals, is a widely prevalent phenomenon in Ricinus. It is also significant that not a single genetically stable female mutant has been found in this extensive survey; therefore, a variant of the Nebraska type must be of extremely rare occurrence in nature.

PHENOTYPIC BEHAVIOR OF SEX-REVERSALS

All sex-reversals begin to bloom as females but sooner or later produce *normal* racemes. Such females may revert following the appearance of the primary pistillate raceme or after bearing over 500 pistillate inflorescences (fig. 1,C–F).

Phenotypic reversion does not occur in all the racemes of a plant simultaneously and some plants remain partly female and partly monoecious for several years. However, the earlier in the life of a female plant a given branch reverts, the sooner will the remaining shoots turn to monoecism. The first reverted raceme may be either normal or predominantly pistillate with a few staminate flowers at the base (fig. 1,B) and this phenotypic change to monoecism is developmentally irreversible. Therefore, femaleness in sex-reversals, or in any branch of a sex-reversal, can be viewed as a developmental phase, of varying duration, which always precedes monoecism as an end-product manifestation. Thus, unlike common fleeting variations, phenotypic reversion is a permanent change which appears to be transmitted lineally during development.

Under conditions favoring strong male tendency, interspersed staminate flowers may be found in the pistillate upper portion of normal racemes as well as in some pistillate inflorescences of sex-reversals. In spring, female racemes of all sex-reversals are strictly pistillate. In summer, interspersed staminate flowers appear intermittently on female racemes of some races of sex-reversals. However, in winter, there is a burst of interspersed staminate flowers on many female inflorescences of the same sex-reversals (fig. 2,E). Naturally, the presence of interspersed staminate flowers in pistillate racemes enables sex-reversals to reproduce themselves even before they revert to normal monoecism. Indeed, certain unstable stocks have already been reproduced in this manner for the past few years.

There exist wide racial differences in the ability to produce interspersed staminate flowers. Sex-reversals of the varieties Adom Mistaef and Queen 162 are quite prone to the production of interspersed staminate flowers. Some of the mutants described by CLAASSEN and HOFFMAN (1950) as Mendelian systems, as well as the interesting mutant described by BLARINGHEM, CHEVET and ROHLFS (1951) are probably similar sex-reversals. On the other hand, the sex-reversals of the variety Gamadon rarely bear interspersed staminate flowers. Whereas sex-reversals are related here to genetic instability, the occurrence of interspersed staminate flowers is a hereditary response to seasonal variations. Previous workers have not been aware of this important distinction between the two phenomena.

BREEDING BEHAVIOR OF SEX-REVERSALS

As pointed out earlier, sex-reversals and some of their monoecious descendants do not breed true. It should be emphasized that, as far as we were able to determine, this hereditary instability is not associated with any impairment of gametic fertility.

The naturally occurring sex-reversals represent a very heterogenous group of plants and they and their descendants can be divided conveniently into two general types, according to their phenotypic and breeding behavior. Type 1 embraces the great majority of the naturally occurring sex-reversals; these usually revert early in life, give, upon selfing, a relatively low percentage of sex-reversals in the offspring, less than 30 percent, and most of their progenies regress rapidly, in the course of a few generations, into stable monoecism. In contrast, type 2 represents rarely occurring sex-reversals that usually revert late in life and give, upon selfing, a relatively high percentage of sex-reversals, about 50 percent, which can be maintained easily and even increased appreciably by selection.

The above division of sex-reversals and unstable stocks into two general types was helpful in the early stages of our investigation and is still of value for characterizing the material used in various experiments. However, with increasing knowledge, we have come to regard all sex-reversals as a single graded series of genetically unstable individuals.

Reproduction of type 1

The breeding behavior of the great majority of naturally occurring sex-reversals is illustrated by the following example. The variety Adamdam, formerly N224A-2-1-3-2, has bred true in our trials to all its visible characteristics including monoecism.

When this inbred was grown on a large scale it proved again to be extremely uniform but consisted of 26 early reverted females in a population of about 40,000 plants. Of the 26 sex-reversals 24 were selfed and their offspring consisted, on the average, of 9 percent sex-reversals, ranging from 0 to 39 percent, based on a total of 899 plants. However, of the 24 progenies 9 consisted of monoecious offspring only. Selfing the remaining 15 lines for two additional generations resulted in a very low incidence in a few families, and in complete disappearance of sex-reversals in the majority. Although it is difficult to develop from such breeding material high sex-reversal producing stocks, it will be shown later that selection, when properly applied, can be effective even in type 1.

Naturally occurring sex-reversals were discovered in Adom Mistaef, Baker, Blackwell, Cimarron, Mautner, N201-2, R-248, U.S. 72 and in other varieties. Most of these females were early-reverted, i.e., they turned to monoecism before bearing their tenth raceme. However, a few exceptional individuals were also observed. These were late reverted females which produced very low percentages of sex-reversals.

Reproduction of type 2

Our first genetically unstable stocks of type 2 originated in two naturally occurring late reverted females found in distinctly different varieties. One mutant was discovered in 1950, in a dwarf variety of Far Eastern origin, and has been named Gamadon. The other mutant was discovered in 1952 in a large plantation of the open pollinated Moroccan variety, Oued Beth; this mutant is known as Queen 162. In contrast to sex-reversals of type 1, each of these two mutants produced over 50 percent of sex-reversals in the immediate offspring and the descendants have in general maintained the ability to produce high percentages of sex-reversals.

The Gamadon mutant was reproduced by selfing for six generations and thus far over 50,000 plants have been grown from it. This mutant breeds true to most visible characteristics, including small morphological structures, habit of growth, five nodes to the primary raceme, short internodes, and plant color. None of these characteristics has been affected during the course of inbreeding. In sex-reversals of Gamadon we have usually selfed normal racemes. However, it is also possible to self in a different manner, i.e., by the transfer of pollen from normal to pistillate inflorescences. In limited tests, no significant differences were noted between these two methods of selfing with regard to the percentage of sex-reversals in the offspring. Sibbing plays an important role in the reproduction of unstable stocks under natural conditions. By sibbing, we mean, in this case, the transfer of pollen from either sex-reversals or monoecious individuals to pistillate racemes of other sex-reversals of the same family. The results of artificial sibbing of this kind do not vary significantly from those obtained from selfing.

The data obtained from selfing of Gamadon for six consecutive generations can be summarized as follows: 1. Sex-reversals do not breed true and they usually differ from each other, in any selfed generation and in any family, in their individual ability to produce sex-reversals in the offspring. Although some sex-reversals are capable of producing 100 percent sex-reversals in their *immediate* offspring, others produce less than 30 percent sex-reversals. 2. Selection of certain individual sex-reversals in each

generation can assure the maintenance of high sex-reversal producing stocks, however, once selection is halted, the percentage of sex-reversals drops steadily in each of the subsequent generations. 3. In any given unstable stock, monoecious individuals produce, on the average, a significantly lower percentage of sex-reversals in the offspring than do sex-reversal plants, but monoecious parents also differ greatly from each other in their ability to produce sex-reversals in the offspring; while some give monoecious offspring only, a few may produce over 50 percent sex-reversals. 4. Stable monoecious lines have been developed from high sex-reversal producing stocks.

Results similar to the above were obtained from selfing Queen 162 and other sex-reversals of type 2. Selfed seed was obtained in Queen 162 by three methods, i.e., selfing pistillate racemes bearing interspersed staminate flowers, selfing normal racemes, and selfing by transferring pollen from normal to pistillate racemes of the same sex-reversal plant. No consistently significant differences were observed among the three methods of selfing, with respect to the percentage of sex-reversals in the offspring.

Time of reversion—basis of selection

During the early stages of this study, in 1951, data were secured which indicated that, in unstable stocks of type 2, a relationship exists between *time* at which reversion occurs in sex reversals and their *ability* to produce sex-reversals in the offspring. However, it soon became apparent that, with the exception of extreme phenotypic categories, the breeding behavior of many sex-reversals of intermediate phenotypes cannot be predicted with a high degree of precision, on the basis of time of reversion. For example, in a given stock of Gamadon, sex-reversals were divided into three phenotypic groups: A. *Early*, females which reverted before the appearance of the tenth raceme; B. *Intermediate*, females which reverted between the tenth and the twentieth raceme; and C. *Late*, females which reverted after bearing over twenty pistillate inflorescences. Groups A (6 plants), B (9 plants), and C (12 plants) produced in their respective selfed progenies 46.4 percent sex-reversals based on 97 individuals, 43.7 percent based on a total of 231 individuals, and 60.4 percent based on 310 plants. Within each group, phenotypically similar individuals may produce significantly different percentages of sex-reversals, but in group C, exceedingly late reverted females, which turn to monoecism after bearing over 100 pistillate racemes, always produce close to 100 percent sex-reversals in their offspring. It is now realized that the above classification into three groups is inadequate since it arbitrarily limits the range of phenotypes in group B and includes in group C an extremely wide range of variants, from females which turn to monoecism between the 20th and the 25th racemes to others which revert after bearing over 500 pistillate racemes! Nevertheless, on the basis of this classification a more consistent correlation was found between time of reversion and breeding behavior when the latter was measured by the percentage of sex-reversals obtained in the F_1 offspring. Crosses were made between pistillate racemes of phenotypically different sex-reversals of groups A, B, and C of Gamadon with the genetically stable monoecious inbred, Early Spineless. The F_1 of group A (22 parents) consisted of 12.4 percent sex-reversals based on a total of 2278 plants, the F_1 of group B (29 parents) consisted of 28.9 percent based on 4058 plants, and the F_1 offspring of group C (17 parents) consisted of 52.2 percent sex-reversals

based on a total of 2104 individuals. A closer examination of data revealed the existence of some differences in breeding behavior between phenotypically similar individual parents, but all exceedingly late reverted females produced close to 100 percent sex-reversals in crossing as they do in selfing.

In terms of years of growth, exceedingly late reverted females are quite variable in regard to time at which they turn to monoecism. It is believed that the hereditary potentialities of exceedingly late reverted females should be measured not by the percentage of sex-reversals obtained in the immediate offspring—which is usually close to 100 percent—but primarily by the rate of decline in the percentage of sex-reversals for a number of generations, following selfing. This has not been done yet. Fortunately, sex-reversals which are female for an extended period—some sex-reversals may not revert during their life span (about 10 years)—can be selfed by interspersed staminate flowers and the "rate of decline" of each may be obtained experimentally.

It has been demonstrated that any monoecious inbred can be converted into a high sex-reversal producing race either directly from a rare occurring late reverted female of type 2, or indirectly from the more commonly occurring sex-reversals of type 1. The conversion of type 1 into type 2 can be accomplished in two or three generations provided, following selfing, a sufficiently large number of sex-reversals is screened. For example, a naturally occurring sex-reversal of the local variety Adom Mistaef produced upon selfing about 5 percent sex-reversals, based on a population of 860 plants. Most of these sex-reversals were early reverted and they produced very low percentages of sex-reversals in the second inbred generation, but there was one tree which reverted after bearing 388 pistillate racemes and the 145 offspring of that tree consisted of 144 sex-reversals and one monoecious individual. In a similar manner, a high sex-reversal producing stock was developed from individuals of the second inbred generation of the sex-reversals of Adamdam. Once a given individual produces, upon selfing, over 50 percent sex-reversals, it is certain that this offspring is of type 2.

Data are presented in table 2 on the effect of selection for early and late occurring reversions in families of four different varieties. These data show that late reverted females give high percentages of sex reversals which are mostly late reverted; and early reverted females give low percentages of sex reversals which are mostly early reverted. The data in table 2 also show that early reverted females of Gamadon produce, on the average, a higher percentage of sex-reversals than phenotypically similar females of other varieties. This strengthens our general contention that Gamadon has a remarkable ability to generate sex-reversals.

It is concluded from the above that the time of reversion is under hereditary control and that in unstable stocks of type 2 the later in life a plant reverts the higher is its potentiality to produce sex-reversals in the offspring.

Inheritance

In crosses between pistillate racemes of sex-reversals and genetically stable races, female and monoecious, the resulting individual F_1's consist of from 0 to 100 percent sex-reversals depending primarily on the particular sex-reversal parent employed, although the influence of the stable variety cannot be ignored, as will be shown later. In a given unstable stock of type 2, crossing *monoecious* plants with stable races gives,

TABLE 2

Selection for and transmission of time of sex reversion in unstable varieties

Parental material		Offspring						
Unstable variety	Phenotypes selfed	Number monoecious plants	Number early reverted females	Number late reverted females	Total sex-reversals	Total number plants	Percent late reverted females	Percent sex-reversals
Adamdam	7 exceedingly late reverted females	12	15	1546	1561	1573	98.3	99.2
	10 early reverted females	860	157	51	208	1068	4.8	19.5
Adom Mistaef	1 exceedingly late reverted female	1	3	140	144	145	96.6	99.3
	7 early reverted females	404	49	6	55	459	1.3	12.0
Gamadon	18 late reverted females	787	—	—	4841	5628	—	86.0
	9 early reverted females	272	—	—	191	463	—	41.3
Queen 162	22 late reverted females	60	13	1184	1197	1257	94.2	95.2
	10 early reverted females	553	33	2	35	588	0.34	6.0

on the average, a significantly lower percentage of sex-reversals in the F_1 than crossing *sex-reversals* with the same stable varieties. It should be mentioned, however, that at least in one case, the F_1 of a cross between a monoecious plant of type 2, Gamadon, and the stable monoecious inbred, Early Spineless, consisted of 19 monoecious and 11 sex-reversal plants. This particular monoecious plant of Gamadon gave upon selfing over 50 percent sex-reversals in the offspring.

In general, heterozygous sex-reversals revert earlier in life than their sex-reversal parents and earlier than the sex-reversal offspring obtained from selfing of these parents. But heterozygous sex-reversals vary greatly in time of phenotypic reversion, as do sex-reversals in self-reproducing stocks, and in crosses between exceedingly late reverted females and stable races, one is likely to find a few exceedingly late reverted females in the F_1. In such crosses, the F_1 invariably consists of over 90 percent sex-reversals. It therefore appears that the phenotype of sex-reversal is dominant over the phenotype of stable races. Is this "dominance" due to gene action or to cytoplasmic transmission?

The discovery of mutant Queen 162, which bears interspersed staminate flowers during some periods of the year, has enabled us to test the nuclear and cytoplasmic hypotheses. Pollinating stable races, female and monoecious, with pollen from interspersed staminate flowers borne on pistillate racemes of exceedingly late reverted females of Queen 162 results in over 90 percent sex-reversals in the F_1. This proves that the factor for sex-reversal can be transmitted fully through male gametes. ZIMMERMAN and PARKEY (1954) reported that crossing females of the backcross line N145-4 with certain varieties gave 100 percent females in the F_1. It is not unlikely that one of the pollen parents employed by these workers was a sex-reversal and so was the F_1.

Paired reciprocal crosses also proved that sex instability is under nuclear con-

trol. These reciprocal crosses were made within pairs of parental racemes. Each pair consisted of one pistillate raceme, bearing interspersed staminate flowers, of a sex-reversal of Queen 162, and one normal raceme of the stable monoecious variety, Kehalhal. Pollen was exchanged within each pair. The F_1 can be readily distinguished from selfs on the basis of plant color; Queen 162 being red non-waxy, Kehalhal green waxy, and the F_1 red waxy. The data from nine such pairs indicated that pollen from interspersed staminate flowers of a given pistillate raceme transmits to the F_1 offspring the factor for sex instability not less effectively than ovules of the same raceme.

The percentage of sex-reversals in backcrosses, F_1 (sex-reversals × stable races) × stable races, and in F_2's is usually very low and it drops rapidly in subsequent generations. Individual F_2 progenies vary from each other and, in general, monoecious F_1 gives a slightly lower percentage of sex-reversals than sex-reversal F_1. The low sex-reversal potential of the majority of the naturally occurring female individuals may be due, among other factors, to heterozygosity.

Pistillate racemes of exceedingly late reverted females of Queen 162 were crossed with the monoecious variety Kehalhal. The F_1 consisted of 93 percent sex-reversals, based on a total of 2,858 individuals, and the F_2 consisted of 26 percent sex-reversals, based on a total of 1,720 plants. Late reverted females of Gamadon were crossed with the monoecious inbred, Early Spineless. The F_1 consisted of 70 percent sex-reversals and the F_2 of about 12 percent (table 3). If the parental sex-reversal is of type 1, the percentage of sex-reversals in the F_2 may be very low, often less than one percent.

TABLE 3

Inheritance of sex-reversal

Cross	F_1			F_2			
	Phenotype	Number plants observed	Number plants selfed	Number monoecious plants	Number sex-reversals	Total number plants	Percent sex-reversals
Sex-reversals, variety Gamadon × Monoecious variety E. Spineless	Monoecious	56	5	107	0	107	0.0
				106	3	109	2.8
				104	3	107	2.8
				92	11	103	10.7
				94	4	98	4.1
	Total for monoecious F_1			503	21	524	4.0
	Sex-reversals	142	7	84	16	100	16.0
				104	5	109	4.6
				92	17	109	15.6
				69	24	93	25.8
				29	12	41	29.3
				60	1	61	1.6
				57	45	102	44.1
	Total for sex-reversals F_1			495	120	615	19.5
	Grand total			998	141	1139	12.4

Crosses were also made between pistillate racemes of late reverted females of Gamadon and the female inbred Nebraska. Of 51 F_1 plants, 38 were sex-reversals and 13 monoecious individuals. All the 51 F_1 plants were selfed. The F_2 obtained from the sex-reversal F_1 consisted of 30 percent females based on 231 offspring and the F_2 obtained from the monoecious F_1 consisted of 28 percent females based on a total of 3,092 plants. A check on the breeding behavior of the F_2 female segregants showed that there were 25 percent females of the Nebraska type and about 4 percent sex-reversals among the entire F_2 population.

The above data suggest that crossing between stable and unstable races hastens regression towards phenotypic and genetic stability, from F_2 on.

FACTORS AFFECTING INCIDENCE OF SEX-REVERSALS

Pistillate racemes of late reverted females of Gamadon were pollinated by a mixture of pollen obtained from our inbred 97, pistillate-staminate ratio 1:14, and from Early Spineless, pistillate-staminate ratio 1:2. The two F_1 offspring can be distinguished by plant color and waxy bloom. The F_1 Gamadon $\times$ 97 consisted of 91 monoecious and 168 sex-reversal plants and the F_1 Gamadon $\times$ Early Spineless consisted of 23 monoecious plants and 192 sex-reversals. The difference between the two F_1 offspring is highly significant, P being less than 0.01.

In the spring of 1952, the variety Oued-Beth was planted on a large scale by the French firm Organico in two ecologically distinct regions of Morocco; in Nouasseur near Casablanca, relatively mild climate and fairly good soils, and in Aghouatim, close to Marrakech, desert climate and submarginal soils. At the time of full bloom, the average pistillate-staminate ratio in the Nouasseur plantation was 1:2 and sex-reversals were found there at a rate of 1:1500, based on about 100,000 plants. In the Aghouatim plantation, the average pistillate-staminate ratio was 1:7 and not a single sex-reversal plant was found among about the same number of plants as in Nouasseur.

Seed of an unstable stock of Queen 162 was sown on November 11, 1954, and again on March 15, 1955, near Hadera, Israel. The winter planting was in full bloom on March 5, 1955, and of 643 plants, 287 were monoecious and 356 were sex-reversals. The spring planting was in full bloom on June 12, 1955 and of 615 plants, 72 were monoecious and 543 were sex-reversals. The difference between the two fields in regard to the incidence of sex-reversals is highly significant, P being less than 0.01. In spite of these results, certain stocks which consist of close to 100 percent sex-reversals are not affected by date of sowing.

It is interesting that factors which favor strong male tendency may also decrease the incidence of sex-reversals in some stocks.

ON THE NATURE OF SEX REVERSION

A number of breeding experiments were conducted in order to obtain some data bearing upon the nature of the phenotypic change in sex-reversals. In one of the tests, both pistillate and emasculated monoecious racemes of individual sex-reversals of Gamadon were pollinated by the monoecious variety Early Spineless. The F_1 obtained from the pistillate racemes consisted, on the average, of 30.3 percent of

sex-reversals, based on a total of 2,081 plants, and the F_1 progeny obtained from the emasculated racemes consisted of only 2.7 percent sex-reversals, based on a total of 1,213 plants.

If the phenotypic change from femaleness to monoecism is associated with a corresponding hereditary change, as the data suggest, how can one explain the fact that selfing monoecious racemes of exceedingly late reverted females of type 2 often gives about 100 percent sex-reversals in the immediate offspring?

Pistillate racemes of five late reverted females of Gamadon were crossed, during winter, with the stable female inbred, Nebraska. Of 164 F_1 plants, 66 percent were sex-reversals. The reciprocal cross was produced, in spring, in an isolation field by planting Nebraska as seed parent, in alternate rows with Gamadon. The monoecious plants and the early reverted females were pulled out from the rows of Gamadon before Nebraska started flowering; thus, the stable female inbred was pollinated by wind-borne pollen carried from the monoecious racemes of late reverted females of Gamadon. The two parents involved in this cross are green non-waxy and the F_1 progeny can always be recognized from selfs by its waxy appearance (gene interaction). Of 781 plants grown from this reciprocal cross, only 6.5 percent were sex-reversals. However, open pollinated (intrabreeding) seed saved from the monoecious racemes of the late reverted females, used as male parents in this cross, produced over 75 percent sex-reversals based on a total 980 plants. If 6.5 percent of sex-reversals in the F_1 corresponds approximately to the percentage of gametes carrying the reversion factor in the monoecious racemes of the sex-reversal parent, we should have expected that selfing or open pollination of such racemes would give about 13 percent sex-reversals instead of 75 percent actually observed.

More comparable data were obtained from a carefully designed paired three-way pollination test. In this test, monoecious racemes of late-reverted females of Gamadon and pistillate racemes bearing a few staminate flowers at the base of Nebraska were employed. Each operation involved a single pair of parental racemes within which three different pollinations were made; reciprocal crosses as well as selfing of the monoecious raceme of the sex-reversal parent. The pooled data obtained from 25 such pairs show that reciprocal crosses between monoecious racemes of sex-reversals and pistillate racemes of the genetically stable females do not differ significantly. However, while the F_1 offspring consisted of 14 percent sex-reversals, based on a total of 697 plants, the offspring obtained from selfing consisted of 75 percent sex-reversals, based on a total of 143 plants. Again, if 14 percent sex-reversals in the F_1 corresponds to the percentage of gametes carrying the reversion factor in the monoecious racemes of the sex-reversal parent one should have expected that selfing such racemes would give about 26 percent sex-reversals instead of 75 percent actually observed.

Another paired three-way pollination test was made, this time involving late reverted females of Gamadon and the monoecious variety Conner; crossing gave less than 2 percent of sex-reversals, while selfing resulted in about 70 percent.

Thus, the data from crossing of sex-reversals suggest that the phenotypic change from femaleness to monoecism is accompanied by a corresponding genetic change while data from selfing do not allude such a change. One may argue that penetrance of sex-reversals in the F_1 is lower than in the offspring obtained from selfing. How-

ever, this argument is untenable because, employing the same stable races, crossing early appearing *pistillate* racemes of exceedingly late reverted females of type 2 often results in close to 100 percent sex-reversals in the F_1.

Any interpretation of the nature of the phenotypic change in sex-reversals must account for the apparent paradox which the above results have brought to light.

HYPOTHESIS

It is evident from the phenotypic and breeding behavior of sex-reversals and their descendants that sex instability in castors represents a case of the "eversporting" or "variegation" phenomenon. The data show that in sex-reversals of unstable stocks the time of change from femaleness to monoecism is largely under nuclear control and the remarkable efficacy of selection for late occurring reversions recalls the action of "mutable loci" as interpreted by McCLINTOCK (1951). In the absence of critical cytogenetic evidence, it is perhaps superfluous to offer a sound interpretation of sex instability in castors. Nevertheless, the observations on the behavior of sex-reversals and the results from various breeding tests, projected in the light of available knowledge, have prompted the presentation of a tentative genetic hypothesis.

In monoecious races, gene *F* (or the genotypic complex controlling monoecism) exerts its full impact extremely early in ontogeny. In sex-reversals, an unstable nuclear factor brings about the suppression of *F* thereby delaying gene impact, inhibiting male potentiality, and resulting in individuals which begin to bloom as females. The nuclear factor mutates into different suppressors varying in degrees of suppression and stability. These mutations are reversible. The suppression of *F* creates a delicate balance between a number of factors, and the *time* at which gene impact occurs depends on the dose of the suppressor, the potential sex tendency of *F*, and non-genetic variations. The higher the degree of suppression of *F*, the less noticeable is the effect of environmental conditions upon the incidence of sex-reversals.

Phenotypic reversion occurs when the suppressed *F* is released and the majority of the cells in the sex differentiating tissue are homozygous for released *F*. The release of *F* may or may not be associated with the loss of the suppressor. A released *F* can be suppressed again and this suppression can be manifested in femaleness if, following fertilization, an effective suppressor is present in a homozygous condition. According to this hypothesis, the expression of *F* is cyclically controlled by the suppressor and, theoretically at least, sex-reversals could breed true if an effective suppressor became stable. The paradox arising from the paired three-way pollination test is resolved by assuming that, in the F_1, sex-reversals $\times$ stable races, zygotes carrying a suppressed *F* from the sex-reversal parent are likely to develop into sex-reversals but comparable zygotes carrying a released *F* are usually monoecious.

The results of crosses between sex-reversals and stable monoecious races may lead one to conclude that femaleness is dominant over monoecism. However, according to our hypothesis, what is dominant or partially dominant in the F_1 is the delay in gene impact.

While the developmental course of sex-reversals is due to suppression and subsequent release of gene *F* by an unstable nuclear factor, other phenotypic, usually

intermittent, changes which occur during development are attributed to non-genetic fluctuations.

SUMMARY

In the castor-oil plant, gene *F*, for monoecism, controls a genetically *stable* series of sex variants ranging from female, *f*, to strongly male inbreds. The sex expression of these strains is subject to non-genetic variations which occur *intermittently* during the development of individual plants.

Sex-reversals occur spontaneously in many natural populations and inbred races. They represent a genetically *unstable* series of females differing in *time* of life at which they turn to monoecism, as well as in *ability* to produce sex-reversals in the offspring. This phenotypic change from femaleness to monoecism is developmentally *irreversible*.

The offspring of sex-reversals consist of varying proportions of sex-reversals and monoecious individuals. In general, early reverted females produce relatively low percentages of females which are mostly early reverted and exceedingly late reverted females produce over 90 percent females which are mostly late reverted. By rigid selection for early or late occurring reversions, any self-reproducing unstable stock can be converted into either low or high sex reversal producing lines. Once selection is halted, a rapid regression occurs towards stable monoecism in the subsequent generations. This indicates that the mutation in question is reversible but that the main direction is towards monoecism.

The data show that the *time* of sex reversion is under nuclear control. However, nothing is known at present about the nature and location of the unstable nuclear material involved.

Although the incidence of sex-reversals in any stock depends largely upon an unstable nuclear material, it is also influenced by genetic and non-genetic factors affecting sex tendency.

A hypothesis is presented to account for sex instability in castors.

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NATURAL MUTATIONS IN INBRED LINES OF MAIZE AND THEIR HETEROTIC EFFECT. II. COMPARISON OF MOTHER LINE VS. MUTANT WHEN OUTCROSSED TO UNRELATED INBREDS[1]

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THE utilization of heterosis in corn was first outlined by SHULL (1909), and the procedures put forth by him are essentially those in practice today. For the past three decades plant breeders have conducted empirical investigations aimed at a more efficient exploitation of this great genetic phenomenon. Improved methods found have resulted in some advancement. The practical aspects of heterosis would be simplified for the breeder if the relative importance of dominance and overdominance could be evaluated, but geneticists interested in gene action would still need additional information.

The experiments described in Part I of this series of papers (SCHULER 1954) involved a comparison of the homozygous (*AA*) genotype versus the heterozygous (*Aa*) genotype on a highly inbred and therefore highly homozygous genetic background. It was demonstrated that the apparent heterotic effects of single loci for some mutants actually may have been due to an unknown number of concealed additional heterozygous loci. The results did not exclude the possibility of overdominance but merely questioned the basic assumption of a single allelic difference. It was pointed out previously that experiments reported in the literature where overdominance was indicated also may have been based on incorrect assumptions regarding single gene differences, since few attempts were made to investigate concealed genetic variability.

It was felt that the same comparison, i.e. *AA* vs *Aa* ought to be extended to, and repeated in, a genetically heterozygous medium by outcrossing both members of a line pair to inbred lines that were not related to motherline and mutant. Single locus overdominance, if existent, may either be of no consequence, or may be potent to a detectable degree, in a system of heterozygous loci.

SINGLETON (1943) in a short note described a semi-dwarf mutant that occurred in an inbred line of sweet corn. When the mutant and the original inbred line were outcrossed to a common inbred parent, the cross involving the mutant in some cases yielded significantly more than the outcross with the original line carrying the dominant factor for the locus under consideration. No data were presented. In a subsequent note (1947) he reported that when inbred lines were extracted from the above mentioned cross, motherline × mutant, variations such as germless seed, brittle

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and virescent were observed. He mentioned that mutations for combining ability had occurred also. No data were given.

SPRAGUE and MILLER (1953) presented preliminary evidence in favor of the relative importance of a dominance type of gene action in corn. Their evidence needs to be substantiated by results from additional cycles of selection. No control of genetic background in the form of homozygosity or homogeneity was feasible because of the nature of the experiment. Extensive experimentation designed to estimate the ratio of dominance to additive genetic variance has been reported in open pollinated varieties of corn as well as in populations derived from crosses of inbred lines by ROBINSON *et al.* (1949 and 1955). No evidence for overdominance in plant and ear height, ear number, ear length and ear diameter was obtained. For yield there was no clear indication of overdominance in the cases involving open pollinated varieties, while F_1 populations derived from intercrossing inbreds showed overdominance or possibly pseudo-overdominance due to linkages. These experiments also did not involve control of all or even most of the genotype but they yield information on average gene action relative to the particular population under study. A total of 52 chromosomes from natural populations of *Drosophila willistoni* were tested for viability of heterozygous lethals by CORDEIRO (1952). Five of these chromosomes were selected by CORDEIRO and DOBZHANSKY (1954) for their apparent superior viabilities of the lethal heterozygotes, which was contrary to the effect of the average of the 52 chromosomes. Outcrosses to a number of stocks and comparing the normal/normal versus the normal/lethal revealed a slightly beneficial effect of the lethal bearing chromosomes. DOBZHANSKY and SPASSKY (1947) made a second or fourth chromosome homozygous in seven lines. Each line was divided into four sublines, two of which were kept homozygous with respect to the second or fourth chromosome and the other two had a "balancing" chromosome introduced into them which served as a crossover suppressor. Furthermore one of each of the two groups received X-ray treatment. The lines were then propagated over 50 generations. The results indicated partial restoration of the vigor and fertility that had been decreased upon making the chromosome homozygous. Their techniques permitted detection of deterioration or improvements in the viability of homozygotes for the chromosomes studied, but not of heterozygotes.

MATERIAL AND METHODS

A total of eight pairs of inbred lines were available. Each pair consisted of an original inbred line or motherline and the identical line except for carrying a homozygous recessive mutant. A brief description of these lines was presented in table 1 of part I (SCHULER 1954) of this study. Each line carried a different mutation. These were as follows: crinkled leaf, narrow leaf, brachytic stalk, green stripe, two dwarf lines and two small seed lines. The two dwarf and small seed mutants were not allelic. The three inbred lines serving as testers were derived by selfing naturally occurring monoploids (CHASE 1952) developed by DR. S. S. CHASE. The first line (D_I) went through two generations of selfing after diploidization of the monoploid. The second line ($D_{I'}$) was identical to the first except for an additional generation of selfing as a diploid. The third tester (D_{II}) was unrelated to the first two, stemming from another genetic source. It had passed through two diploid generations. The three tester

inbreds are symbolized by D_ID_I, $D_{I'}D_{I'}$ and $D_{II}D_{II}$, where the capital D stands for the respective dominant allele and the Roman subscript for the tester source. Similarly the mutant line pairs may be designated as D_MD_M and d_Md_M, where D and d stand for the gene under study and the subscript M indicates that the allele came from the line pair.

Each of the eight line pairs was grown in 1953 in a single row and used as male parent in an outcross to the unrelated tester inbreds. No attempt was made to resort to the same male plant in each row as pollen sources for all outcrosses, nor was a bulked pollen sample taken consistently. The first six of the above enumerated mutants and their motherlines were outcrossed to all three inbred testers. Due to different maturities the two pairs involving the small seed mutation could not be crossed to tester $D_{II}D_{II}$. These crosses resulted in six F_1 populations (D_MD_I, $D_MD_{I'}$, D_MD_{II}, d_MD_I, $d_MD_{I'}$ d_MD_{II}) for the first six mutants, and four others (D_MD_I, $D_MD_{I'}$, d_MD_I, $d_MD_{I'}$) for the two small seed mutants.

The randomized complete block design adopted was such that each line pair was treated as a separate experiment allowing statistical treatment of intra-line pair comparisons only. The six and four entries respectively of each experiment, were grown in ten replications in 1954 near Ames, Iowa. Each plot consisted of a single row with ten plants spaced approximately 13 inches apart. Four individual plants, competitive plants whenever possible, were taken at random and the following measurements recorded: plant height in centimeters, kernel row number, ear length in millimeters, ear diameter in millimeters, weight of 100 seeds in grams, weight of shelled grain in grams and weight of unshelled ears in grams. Lodging prevented the collection of meaningful data on plant height in the narrow leaf experiment.

For each attribute within each experiment an analysis of variance of the following pattern was calculated:

Analysis of variance

Source	*d.f.*	*E(M.S)*
Total	$srt - 1$	
Replications	$r - 1$	
Treatments (crosses)	$t - 1$	$\sigma_e^2 + s\,\sigma_{rt}^2 + rs\,\sigma_t^2$
Repl. × treatments	$(r - 1)(t - 1)$	$\sigma_e^2 + s\,\sigma_{rt}^2$
Sampling	$(s - 1)\,rt$	σ_e^2

where r represents the number of replications, t of treatments and s of samples (plants within plots). The treatment sums of squares were divided into $t - 1$ orthogonal comparisons:

Symbol	*Description*
C_1	Homoz. dominants vs. heteroz. = $(D_MD_I + D_MD_{I'} + D_MD_{II}) - (d_MD_I + d_MD_{I'} + d_MD_{II})$
C_2	Tester D_I vs tester $D_{I'}$ = $(D_MD_I + d_MD_I) - (D_MD_{I'} + d_MD_{I'})$
C_3	Tester $D_I + D_{I'}$ vs tester D_{II} = $(D_MD_I + d_MD_I + D_MD_{I'} + d_MD_{I'}) - (2D_MD_{II} + 2d_MD_{II})$
I_1	Interaction $C_1 \times C_2$ = $(D_MD_{I'} - D_MD_I) + (d_MD_I - d_MD_{I'})$
I_2	Interaction $C_1 \times C_3$ = $(2D_MD_{II} - D_MD_I + D_MD_{I'}) + (d_MD_I + d_MD_{I'} - 2d_MD_{II})$

The comparisons C_3 and I_2 were not available in the two experiments which had 4 treatments. For testing the variance ratios for treatments and individual comparisons the replication × treatment interaction mean square was used.

Each sampling sum of squares was partitioned into as many parts as there were treatments to obtain information on within treatment variances and ratios of such mean squares.

EXPERIMENTAL RESULTS

The means of the six (four) F_1 populations for each of the eight mutants obtained from the eight experiments are presented in table 1. The variance ratios testing the differences among these means are presented in table 2. Differences generally were significant with the exception of the small seed 1373 line pair. Upon examination of table 1 it becomes obvious that the high F ratios in most cases were due largely to the unlike effect of the tester parent I and I′ versus II. This result was anticipated. Table 3 contains the variance ratios for the orthogonal comparisons. Under each F value the linear combination obtained by adding and subtracting the treatment totals according to the orthogonal linear forms is placed in parentheses. Table 3 reveals the main points of the experiments.

The results can be viewed in terms of individual line pairs, of attributes, or of orthogonal comparisons over all line pairs. A brief description of the results obtained for each line pair may suffice.

The experiment involving the crinkled lines gave significant variance ratios for all attributes (table 2). This also was the case for contrasts C_3 of table 3, indicating a differential combining ability among the two tester sources. The direction of these differences varied from one trait to the next. The comparison C_1, which was considered the most important aspect of the experiments, indicated that the recessive crinkled allele when combined with the dominant was superior to the homozygous dominant genotype for plant height, kernel row number and ear length but inferior for ear diameter. Yields did not differ significantly. The comparison C_2 was chosen to reveal and test differences between tester I and I'. In no case were variance ratios high. Only one of the interactions gave a significant F ratio.

The experiments with the narrow leaf and green stripe gave no discrimination between the D_MD_T and d_MD_T genotypes (T stands for Tester). Significance was obtained between the two tester sources (C_3). Of the few significant F values in the brachytic line experiment, kernel row number was significant in comparison C_1, C_3 and I_2. The results of the two dwarf mutants were similar in that in most attributes the differences between testers were significant (C_3) and in some instances also the comparison involving homozygous dominants versus the heterozygotes. The only cases in all of the experiments in which D_MD_T vs d_MD_T differed in yield were in the dwarf SW1, dwarf 187-2 and the small seed M14 mutant. However, the homozygous dominant genotype exceeded the heterozygous genotype for both dwarf mutants and the small seed M14 mutant. The two testers I and I′ differed significantly in the small seed M14 test for plant height, ear diameter and both yield measurements. Furthermore, some interactions were significant.

The mean squares on a per plant basis for sampling variances are too bulky for

TABLE 1

Means for each measured attribute in four or six crosses with each of eight mutants

Mutant	Genotype	Plant height	Kernel row number	Ear length	Ear diameter	100 kernel weight	Weight of shelled grain	Weight of unshelled grain
cr	$d_M D_I$	228.27	18.35	242.92	46.10	34.75	233.35	272.20
	$d_M D_{I'}$	225.15	17.70	246.15	45.90	34.62	231.25	271.50
	$d_M D_{II}$	238.67	20.55	227.15	49.30	32.87	259.65	296.95
	$D_M D_I$	217.03	17.05	232.25	47.35	35.50	245.75	285.37
	$D_M D_{I'}$	215.10	16.70	233.75	47.00	35.47	228.80	266.12
	$D_M D_{II}$	235.77	19.80	222.02	51.12	33.60	268.10	303.85
nl	$d_M D_I$		14.00	200.15	43.62	34.05	187.47	219.17
	$d_M D_{I'}$		14.35	198.70	43.67	31.50	180.75	208.70
	$d_M D_{II}$		17.40	200.47	47.20	31.15	211.60	240.80
	$D_M D_I$		14.65	201.27	44.12	33.32	185.30	215.90
	$D_M D_{I'}$		14.45	198.00	43.87	32.55	172.05	201.80
	$D_M D_{II}$		17.50	197.55	46.52	31.07	193.20	222.27
br	$d_M D_I$	182.67	18.55	215.25	48.40	30.67	214.80	254.97
	$d_M D_{I'}$	185.40	18.40	207.65	47.70	28.97	196.75	238.50
	$d_M D_{II}$	203.77	19.05	212.67	47.67	28.65	209.42	246.52
	$D_M D_I$	188.25	17.90	210.57	47.60	30.47	205.52	248.62
	$D_M D_{I'}$	189.40	18.20	206.02	47.62	29.60	201.00	243.90
	$D_M D_{II}$	202.77	21.55	213.65	48.97	28.10	205.35	249.05
gs	$d_M D_I$	200.25	16.75	230.30	48.30	27.30	191.85	233.35
	$d_M D_{I'}$	202.37	17.10	214.97	48.77	28.72	207.00	251.25
	$d_M D_{II}$	215.45	22.15	207.90	51.32	27.05	236.57	273.62
	$D_M D_I$	208.00	17.90	220.85	48.52	25.45	196.37	238.62
	$D_M D_{I'}$	204.85	17.00	228.02	48.07	27.42	215.62	256.60
	$D_M D_{II}$	211.70	20.35	204.62	49.92	27.10	232.65	272.90
d SW1	$d_M D_I$	181.05	17.10	215.30	47.17	29.95	219.00	253.82
	$d_M D_{I'}$	175.75	16.85	221.80	47.15	29.65	205.52	238.57
	$d_M D_{II}$	219.77	19.20	227.57	49.02	30.70	247.07	283.77
	$D_M D_I$	217.72	16.15	225.62	48.22	31.85	226.55	270.95
	$D_M D_{I'}$	219.57	15.65	228.75	48.27	32.92	216.90	263.22
	$D_M D_{II}$	236.32	18.45	218.92	50.17	30.87	259.25	302.25
d 187–2	$d_M D_I$	226.07	16.45	235.37	46.40	29.47	232.52	269.02
	$d_M D_{I'}$	226.42	17.00	238.97	47.92	30.67	246.50	287.72
	$d_M D_{II}$	237.20	21.20	221.42	49.70	27.47	248.15	278.00
	$D_M D_I$	218.85	15.70	239.25	47.32	32.02	256.55	293.55
	$D_M D_{I'}$	214.57	16.00	238.52	47.50	30.95	244.50	282.80
	$D_M D_{II}$	239.27	19.95	231.57	49.67	38.67	267.65	298.92
sm M14	$d_M D_I$	193.05	15.75	193.07	43.90	29.60	160.82	189.25
	$d_M D_{I'}$	206.10	16.80	233.00	46.37	30.55	216.77	253.82
	$D_M D_I$	206.92	16.70	232.97	46.42	30.20	214.87	250.65
	$D_M D_{I'}$	205.37	16.50	223.00	46.52	30.32	215.67	252.75
sm 1373	$d_M D_I$	222.82	15.30	221.20	44.40	32.07	206.82	242.55
	$d_M D_{I'}$	221.32	15.50	220.55	45.37	32.05	220.30	258.67
	$D_M D_I$	220.15	15.75	220.62	45.12	32.20	215.15	251.35
	$D_M D_{I'}$	219.35	15.00	213.32	44.82	31.87	191.05	225.42

TABLE 2

Variance ratios from the analyses of variance of the attributes measured in eight mutants

Mutant	Plant height	Kernel row number	Ear length	Ear diameter	100 kernel weight	Weight of shelled grain	Weight of unshelled grain
cr	12.88**	40.78**	7.08**	21.85**	2.90*	4.05**	2.67*
nl		40.47**	.24	14.12**	3.94**	3.69**	2.62*
br	11.46**	42.63**	1.60	1.72	2.01	.66	.39
gs	6.51**	6.56**	1.93	4.01**	2.12	5.58**	3.42*
d SW1	62.51**	21.90**	1.09	8.97**	5.31**	7.73**	6.83**
d 187-2	9.19**	129.63**	3.27*	12.91**	6.49**	2.34	1.65
sm M14	9.74**	3.59*	6.34**	4.82**	1.20	6.00**	5.95**
sm 1373	.40	.73	1.45	1.72	.05	2.97	2.75

* Significant at the 5% level.

** Significant at the 1% level.

inclusion (a copy is on file in the Genetics Editorial Office). The values indicate that sampling variances in general were homogenous. No statistical test for homogeneity of variance was made since no simple and satisfactory method was available and BARTLETT's test for homogeneity was known to be sensitive for non-normality. Variance ratios of the form of $D_MD_{I'}/D_MD_I$ and $d_MD_{I'}/d_MD_I$ may shed some light on the problem of the observed increase of the plant to plant variability in successive generations of diploidized monoploids. These variance ratios were one-tail tests with $F = 1.84$ and 2.38 at the 5 percent and 1 percent level of probability respectively. All ratio tests were based on 30 and 30 degrees of freedom and are presented in table 4.

The test involving the brachytic and small seed M14, and to a lesser extent the dwarf 187-2 and green stripe line pairs gave some significant deviations indicating a difference between the two tester lines I and I′. The variance ratios intended to reveal differences in the genetic composition of mutants and motherline seemed to have no consistent trend. An accumulation of significant values in the crinkled test for tester II and the small seed M14 test for tester I was noted.

DISCUSSION

In part I of this series the original inbred line was crossed with its counterpart which was thought to be genetically identical except for a mutant allele. Genetic identity, which requires complete homozygosity could not be met, however. Heterozygosity persisted even after many generations of inbreeding. Explanations as to the source of such remnant heterozygosity have been put forth, but which of the possible forces are important remains to be demonstrated. Very likely mutations may create variability but other factors such as selective bias in favor of more heterozygous individuals, the pure chance in inbreeding coupled with the number of loci involved, the mechanical procedures used by breeders or as yet unrecognized phenomena may account for such variability. Remnant heterozygosity in connection with the control of the genetic medium may have a substantial effect if both homozygosity and homogeneity of genotypes are the critical assumptions. However, it

TABLE 3

The magnitude of the differences and the variance ratios for all attributes measured in three, or four orthogonal comparisons among the four or six crosses with eight mutants

Mutant	Comparison	Plant height	Kernel row number	Ear length	Ear diameter	100 kernel weight	Weight of shelled grain	Weight of unshelled grain
cr	C_1	13.70**	26.85**	11.15**	15.37**	2.39	.86	.41
		(−968)	(−122)	(−1128)	(167)	(93)	(736)	(588)
	C_2	.89	4.33*	.47	.40	.02	1.38	1.13
		(202)	(40)	(−189)	(22)	(6)	(762)	(798)
	C_3	46.92**	171.44**	22.56**	92.70**	12.08**	17.11**	10.78**
		(−2534)	(−436)	(2269)	(−580)	(296)	(−4654)	(−4256)
	I_1	.05	.39	.06	.03	.01	.84	.98
		(48)	(12)	(−69)	(−6)	(4)	(−594)	(−742)
	I_2	2.81	.92	1.15	.74	.01	.06	.03
		(620)	(32)	(513)	(52)	(−6)	(278)	(240)
nl	C_1		1.88	.11	.00	.03	2.94	2.01
			(34)	(−180)	(1)	(10)	(−1171)	(−1148)
	C_2		.09	.61	.00	7.14*	2.05	2.21
			(−6)	(189)	(8)	(133)	(799)	(983)
	C_3		198.60**	.04	68.53**	10.47**	12.10**	7.93*
			(−494)	(83)	(−486)	(279)	(−3361)	(−3223)
	I_1		1.18	.09	.13	2.03	.22	.05
			(−22)	(−73)	(−12)	(71)	(−261)	(−145)
	I_2		.39	.36	1.95	.05	1.15	.88
			(−22)	(−251)	(−82)	(−19)	(−1037)	(−1075)
br	C_1	1.72	10.87**	.59	.16	.00	.23	.01
		(343)	(66)	(−213)	(17)	(−5)	(−364)	(63)
	C_2	.53	.96	4.61*	.61	3.18	2.13	1.47
		(−155)	(−16)	(486)	(27)	(103)	(903)	(848)
	C_3	53.38**	132.58**	1.80	1.75	6.19*	.18	.03
		(−2695)	(−326)	(−526)	(−79)	(249)	(−459)	(−206)
	I_1	.08	1.21	.29	.71	.33	.76	.45
		(−63)	(18)	(122)	(29)	(33)	(541)	(470)
	I_2	1.58	68.31**	.71	5.43*	.37	.01	.04
		(−463)	(234)	(330)	(139)	(−61)	(125)	(240)
gs	C_1	1.37	.13	.00	1.52	3.10	.23	.20
		(259)	(−30)	(13)	(−75)	(−124)	(369)	(369)
	C_2	.05	.10	.30	.00	5.60*	4.83*	3.89
		(41)	(22)	(326)	(−1)	(−136)	(−1376)	(−1435)
	C_3	24.62**	29.63**	7.02*	16.78**	.05	22.17**	12.90**
		(−1553)	(−650)	(2764)	(−353)	(24)	(−5104)	(−4529)
	I_1	1.36	.53	2.23	.55	.15	.07	.00
		(−211)	(−50)	(900)	(−37)	(22)	(164)	(3)
	I_2	5.13*	2.43	.16	1.16	1.71	.60	.20
		(−709)	(−186)	(−406)	(−93)	(130)	(−840)	(−483)
d SW1	C_1	167.15**	16.69**	.43	12.47**	17.01**	3.05	8.21**
		(3882)	(−116)	(−276)	(133)	(214)	(1244)	(2410)

TABLE 3—*continued*

Mutant	Comparison	Plant height	Kernel row number	Ear length	Ear diameter	100-kernel weight	Weight of shelled grain	Weight of unshelled grain
	C_2	.31	1.67	.48	.00	.54	2.53	1.79
		(138)	(30)	(236)	(−1)	(31)	(925)	(919)
	C_3	123.76**	90.48**	.89	32.36**	.45	32.96**	23.94**
		(−4724)	(−382)	(560)	(−303)	(49)	(−5787)	(−5819)
	I_1	1.36	.18	2.02	.01	1.69	.07	.19
		(286)	(−10)	(486)	(3)	(55)	(153)	(301)
	I_2	19.93**	.42	1.65	.00	6.92*	.05	.03
		(−1896)	(26)	(−762)	(5)	(−193)	(217)	(−193)
d 187-2	C_1	4.64*	36.42**	2.11	.27	6.36*	5.01*	3.86
		(−680)	(−120)	(543)	(19)	(161)	(1701)	(1621)
	C_2	.37	4.46*	.14	5.23*	.01	.00	.22
		(157)	(−34)	(−115)	(−68)	(−5)	(−37)	(−318)
	C_3	36.09**	605.19**	12.18**	55.55**	23.00**	3.54	.50
		(−2681)	(−686)	(1845)	(−384)	(433)	(−2021)	(−830)
	I_1	.51	.38	.32	3.29	3.04	3.04	3.06
		(−185)	(−10)	(−173)	(−54)	(−91)	(−1081)	(−1178)
	I_2	4.33*	1.16	1.63	.18	.03	.35	.58
		(929)	(−30)	(675)	(−22)	(−17)	(639)	(890)
sm M14	C_1	9.75**	1.11	3.97	5.34*	.20	5.56*	5.42*
		(526)	(26)	(1196)	(107)	(15)	(2118)	(2413)
	C_2	7.46*	1.90	3.98	4.95*	2.08	6.38*	6.62*
		(−460)	(−34)	(−1198)	(−103)	(−43)	(−2270)	(−2667)
	I_1	12.02**	4.10	11.06**	4.21*	1.23	6.04*	5.81*
		(−584)	(−50)	(−1996)	(−95)	(−33)	(−2206)	(−2499)
sm 1373	C_1	.75	.00	.36	.08	.00	1.99	1.99
		(−186)	(−21)	(−312)	(7)	(−2)	(−837)	(−978)
	C_2	.23	1.03	1.63	1.12	.11	.51	.32
		(2)	(22)	(318)	(−27)	(14)	(425)	(392)
	I_1	.02	3.08	1.14	4.00	.08	6.40*	5.87*
		(28)	(−38)	(−266)	(−51)	(−12)	(−1503)	(−1682)

* Significant at the 5% level.
** Significant at the 1% level.

might carry less weight if the contrast *DD* vs *Dd* is made on a highly heterozygous genetic background as was accomplished in this experiment by taking unrelated inbreds as tester parents.

The Drosophila experiment of CORDEIRO (1952) and others, where normal/normal was compared with the normal/lethal, deviated from this approach in that inferences about viability effects of a heterozygous lethal depended on the assumption of random genetic background. Results obtained with Drosophila may be further complicated by linkage effects. The latter limitation seems especially to impair conclusions about single locus heterosis in Drosophila. Even though some of these handicaps can be overcome with maize, it is unlikely that one might, beyond doubt,

TABLE 4

Ratios of within-cross variance for eight line pairs and seven attributes

Mutant	Variance ratio	Plant height	Kernel row number	Ear length	Ear diameter	100 kernel weight	Weight of shelled grain	Weight of unshelled grain
cr	$D_MD_{I'}/D_MD_I$	1.38	1.05	1.07	.66	1.05	1.00	1.10
	$d_MD_{I'}/d_MD_I$	.80	.64	1.14	.94	.75	1.44	1.38
nl	$D_MD_{I'}/D_MD_I$		1.17	1.80	.98	.69	.72	.70
	$d_MD_{I'}/d_MD_I$		1.50	2.77**	1.24	3.85**	.61	.60
br	$D_MD_{I'}/D_MD_I$	.65	1.08	3.62**	2.62**	1.70	4.40**	4.53**
	$d_MD_{I'}/d_MD_I$	.67	.90	3.24**	5.03**	4.82**	3.23**	4.81**
gs	$D_MD_{I'}/D_MD_I$	1.48	.80	1.07		.45	.61	.71
	$d_MD_{I'}/d_MD_I$	2.60**	1.50		1.60	1.43	2.28**	2.51**
d SW1	$D_MD_{I'}/D_MD_I$	1.05	2.02*	.69	1.04	.91	1.03	.92
	$d_MD_{I'}/d_MD_I$	1.39	.67	1.58	1.30	.59	2.21	2.05*
d 187-2	$D_MD_{I'}/D_MD_I$	.95	.60	1.36	1.11	1.44	.68	.74
	$d_MD_{I'}/d_MD_I$	.93	.80	.68	2.23*	.59	2.31*	2.31*
sm M14	$D_MD_{I'}/D_MD_I$	.60	1.58	4.12**	4.80**	1.38	2.87**	3.05**
	$d_MD_{I'}/d_MD_I$	.24	.34	.36	.50	1.00	.57	.53
sm 1373	$D_MD_{I'}/D_MD_I$	1.40	.89	.83	.93	1.67	.77	.58
	$d_MD_{I'}/d_MD_I$	.84	1.39	.67	.40	.95	1.12	1.15

* Significant at the 5% level.
** Significant at the 1% level.

secure two isogenic lines that differ by one allele only. Barring pseudo-overdominance effects of linkage, the need for complete isogenicity may be evaded. The following proposal for future work is advanced. Two highly isogenic lines differing by one conspicuous mutation are crossed and advanced to the F_2 or F_n generation to allow recombinations. This would tend to smooth out any effects of additional heterozygous loci and their interactions. The two genotypes *DD* and *Dd* would have an equal chance to combine with either allele of any other heterozygous locus. Such studies already are under way.

The data obtained on shelled and unshelled grain as a measure of yield did not support the hypothesis of overdominance. No significant *F* value obtained for yield favored the *Dd* genotype. These results were consistent for all mutants regardless of their behavior when tested on a highly homozygous background. If overdominance existed it was of such small effect in terms of the total heterosis that it could not be measured. These results are contrary to those obtained for the one mutation reported by SINGLETON (1943). However, in a later note (1947) he mentioned that mutations for combining ability also had occurred.

It is conceivable that many moderate overdominance effects on a number of loci, if cumulative, could be of determinable magnitude and therefore overdominance, unimportant on a single locus, may be of consequence because of its additive effects between loci. If this were the case one would expect results contrary to those reported by ROBINSON *et al.* (1949, 1955) and SPRAGUE and MILLER (1953).

No other attribute gave as consistent a picture as yield. For example in kernel row number the *Dd* genotype exceeded the *DD* class in the crinkled, dwarf SW1 and

dwarf 187-2 experiments, while the reverse was true for the brachytic test. Ear diameter was significant in the *DD* plants in two tests, in the *Dd* plants in one, and non-significant in the remainder.

In terms of individual experiments, the tests involving crinkled had three attributes significantly superior in the *Dd* class and in one the *DD* class was the better. In the small seed M14 test the homozygotes consistently exceeded the heterozygotes and the *F* value for plant height, ear diameter and yields had a probability of less than 5 percent. Small differences for all attributes were found for the green stripe, narrow leaf and small seed 1373 tests. The former (green stripe) was classified in the previous paper as a possible overdominant locus, while for the latter two no such effects were postulated. Those results seem to support the impression that with respect to this sample of loci, single locus heterosis effects if present were of no weight in a heterozygous genetic medium. Possible exceptions such as crinkled may need further study, although for yield the conclusions hold here as well.

The problem of heterozygosity remaining after many generations of selfing is widely recognized among plant breeders. It is tied directly into the assumptions employed in the preceding study and to a lesser extent in the experiment reported here. Inbreds derived by diploidizing monoploid plants are phenotypically extremely uniform but in succeeding generations this uniformity seems to deteriorate to such a degree that phenotypic plant to plant variance is very noticeable. Such an increase in variability within double monoploid inbred lines, if real, can not be explained by relic heterozygosity but must have originated in the homozygous gene complex by mutational changes. This observation by the writers and other workers in the field has not been evaluated in experimental trials. If subsequent data substantiate the observation then our present opinion on mutation rates, mutation effects, etc. may need to be revised before extending it to multigenic metric characters. Tests are now under way to determine whether an increase in variability occurs to a measurable degree.

Because little concrete information is available it was felt that by taking individual plant data, the within-cross variances and their ratios so obtained, while not the primary objective of the experiment, might yield some supplementary information. The results are summarized in table 4. Deficient and preliminary as they may be, they indicate nevertheless that plant to plant variance and variance between motherline and mutant could be critical factors. Furthermore, occasional *F* ratios of comparison C_2 in table 3 reached significant levels. However, it would be rather surprising if one additional sexual cycle gave rise to enough mutations for combining ability so as to result in a constantly altered performance of the line. Investigations aimed at an explanation of the genetic causes of variability within inbred lines are needed for the breeder of naturally and artificially self-pollinated crops as well as for future research. A logical extension of such analyses would lead to an examination of the magnitude of variances among homozygous and/or homogeneous plants in adapted and unadapted environments and of genotype-environment interaction.

SUMMARY

1. The homozygous dominant genotype (*DD*) was compared with the heterozygous genotype (*Dd*) in a genetically heterozygous but homogeneous background. Eight

inbred lines and their sub-strains characterized by a homozygous recessive mutant were crossed onto three testers that had been obtained by doubling monoploids. The crosses were grown in eight separate randomized block designs. Pertinent orthogonal comparisons were calculated in the analyses. Sampling variances and ratios of such variances were presented.

2. Without exception there was no evidence of overdominance for yield. On other attributes occasionally the heterozygotes exceeded the homozygous dominants. Variance ratios indicated that genetic homogeneity was not a valid assumption for all mutants or all attributes. Causes of heterozygosity in inbred lines were discussed.

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HETEROSIS IN THE HONEY BEE (APIS MELLIFERA L.)

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THERE are three castes in the honey bee colony: the drone, queen, and worker. The latter two are both females. The drone bee is a male, representing a haploid gamete of the queen bee heading his hive. The honey bee colony is composed of two generations of bees. The queen bee is, in effect, a potent egg-laying machine, while the workers are morphologically and functionally fitted for honey and pollen gathering and for most other work of hive and field, except that under normal circumstances they do not lay eggs. The queen bee is the parent mother of the colony, and the worker bees are her progeny.

Breeding methods to insure heterosis have led to increased vigor and productivity in corn, sorghum, Drosophila, poultry and other species. These species are diploid in both sexes. It is of interest to examine another species, having a different system of reproduction, for heterotic effects and to analyze for the responsible mechanisms. In this study hybrid vigor is regarded as the superiority of the hybrid over the better parent. The characters chosen for study are egg production of the queen, a character largely dependent on the queen's own genotype, and honey yield, a character dependent on the vigor and well being of the queen's worker progeny. Honey yield consequently differs from egg production in being influenced by the genome of the drones to which the queen is mated. The single queen will contribute a genome to each of the 50 or 60 thousand workers making up the colony.

In the past the honey bees must have been intensely selected for the capacity to produce large numbers of progeny and workers active in gathering food supplies. The individual worker bee lives about 6 weeks during the active summer season. Rapid replacement of this worker force depends on the egg laying of the single queen mother. For existence the colony must harvest sufficient nectar in six to ten weeks to last the balance of the year. Till recently, numbers for egg laying and colony replacements have been rather vague, 200 to 5000 eggs per day were estimated. NOLAN (1923) has presented data indicating that rates of less than 2000 eggs per day over a 12 day period were to be expected. Larval deaths will reduce the effective worker groups. The effective oviposition rate will be less than the observed eggs laid, the actual oviposition, by any genetic or other factors favoring the death of the larva. In the inbreeding necessary to the formation of the inbred lines utilized in this study hypothesized sex alleles could be factors in this loss of larvae. MACKENSEN (1951), using brood viability counts, has postulated for the honey bees a series of haploviable alleles which are lethal in homozygous diploid females. However, earlier work of CALE (1952) indicated that the oviposition rate of inbred queens was independent

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of percentage inbreeding in the range 31 to 77 percent. The queens utilized in this study were observed to have high egg viability prior to collection of the data.

MATERIALS AND METHODS

The genetic material for this study was composed of four inbred lines of honey bees. Following an original selection, no further selections were practiced during the inbreeding of any of these lines. No precise method of inbreeding was used in establishing and continuing any of the lines. Selection of male and female parents from one generation to the next within each strain was more often determined by the availability of males than by attempting to retain a strict parent-offspring or brother-sister system of inbreeding. The coefficients of inbreeding for the four lines at the initiation of these experiments were as follows: D, 56.7%; F, 54.6%; H, 65.8%; J, 64.8%. All matings in the formation of these inbred lines were instrumental inseminations, the sperm being gathered from several suitable males.

Eleven groups of queens and the honey producing capabilities of their worker progeny were studied. These groups included the following: inbreds D, F, H, and J; F_1 females representing all possible non-reciprocal single crosses of the four inbred lines; and a random group representing the available so-called line-bred stock obtained from the queen breeders of this country. The four inbred lines and their six single cross F_1's were all daughters of instrumentally mated queens.

It was considered impractical to inseminate instrumentally the inbred and F_1 females chosen for study. Each queen would have needed at least two inseminations. This would have meant more than 500 artificial inseminations—a task beyond the limits of the equipment and personnel available. These queens were allowed to fly from small nuclei colonies and mate with whatever drones they encountered. TABER (1954), using a genetic marker called cordovan, has indicated that queen honey bees allowed free flight mate on an average six and one-half times. This fact is considered important in view of the assumption of random contribution of the males to the yield potential of the worker progeny.

Queens were reared and mated in the summer of 1953. They were then randomly assigned to the test apiaries and introduced to the bee colonies in the fall of 1953. In the spring of 1954 each apiary, or replication, was handled as a unit. Within the apiary, any necessary adjustment of pollen supply, honey, or bees was confined to adjustments within—rather than between—groups being studied.

Only one determination of effective oviposition rate was made, since earlier work indicated that one count of oviposition rate based on large numbers would show real differences (CALE 1952). The oviposition rate determined was a 12-day average preceding the honey flow by 10–22 days. The technique for this determination was fully described in the above citation.

The data on yield were collected at the end of the honey flow period. Each of the 247 colonies was weighed and a record made of the total weight of the colony as well as the number of hive units represented for each colony. The total yield of honey was obtained by subtracting the weight of the hive units from the total weight of the colony.

For the purposes of this study, general combining ability was defined as the aver-

TABLE 1

Average productivity expressed as eggs per day and pounds of honey by breeding group and replication

Replicate	F_1 females											
	H × F		J × F		D × H		J × H		J × D		D × F	
	Eggs	Honey	Eggs	Honey	Eggs	Honey	Eggs	Honey	Eggs	Honey	Eggs	Honey
1	1539	308	1596	252	1145	228	1353	282	1664	243	1384	243
2	1365	186	1341	156	1078	191	1378	225	1520	204	1149	203
3	1672	254	1268	172	1264	167	1695	259	1609	245	1591	221
4	1345	193	1474	199	1148	154	1569	208	1468	162	1101	138
5	1208	181	1324	195	1401	195	1524	284	1523	202	1276	191
6	1638	288	1554	301	1215	259	1507	281	1422	243	1444	207
Averages	1461	235	1426	213	1208	199	1504	256	1534	216	1324	201

	Inbred females								Randomly mated females	
	J		D		F		H			
	Eggs	Honey	Eggs	Honey	Eggs	Honey	Eggs	Honey	Eggs	Honey
1	836	234	830	239	1140	243	1216	281	1423	239
2	851	144	986	151	1160	200	1201	235	1310	200
3	678	114	891	114	1110	146	1343	162	1368	217
4	773	173	766	121	793	133	1163	180	1167	177
5	1107	137	959	148	666	165	1103	147	1305	190
6	1015	248	1107	152	877	206	817	189	1313	223
Averages	876	175	922	154	957	182	1140	199	1314	207

age performance of a line in contrast to the average of the group, specific combining ability as the performance of the specific cross in relation to the average performance of the lines which were the parents of the cross.

EXPERIMENTAL RESULTS

The mean oviposition rates and honey yields by group and replicate have been assembled in table 1. Inbred H had the greatest degree of inbreeding, and also had the highest 12-day oviposition rate of the four inbreds studied—1140 eggs per day. The next highest oviposition rate, 957 eggs per day, was associated with inbred F, the inbred having the least percentage inbreeding. It was indicated previously that oviposition rate was practically independent of the percent of inbreeding which had taken place in the formation of lines. These figures confirmed this fact. For all four inbreds, the range of oviposition rate was from 876 eggs per day for line J, to 1,140 eggs per day for line H. The average of the inbreds for the period under consideration was 974 eggs per day.

The F_1 females yielded mean oviposition rates ranging from 1,208 to 1,534 eggs per day, with an average for all six F_1's of 1,409 eggs per day. Measurements of the productivity of the F_1's were from 68 to 658 eggs per day greater than similar measurements of the parental inbreds.

TABLE 2

Sources of variation and their contributions to variations in oviposition rate and honey yield

Source	Oviposition rate		
	d.f.	M.S.	*F*
Locations	5	35220.1	1.7
Groups	10	345641.9	16.5**
Random & parents versus hybrids	1	2208936.3	105.6**
Random versus parents	1	554987.7	26.5**
Parents	3	80270.7	3.8*
Hybrids	5	90336.7	4.3**
General	3	85197.7	4.1*
Specific	2	98045.3	4.7*
Error	50	20925.1	
Total	65		
	Honey yield		
Locations	5	12504.9	33.2**
Groups	10	4706.5	12.5**
Hybrids versus random	1	16377.8	43.5**
Inbreds versus random	1	12828.7	34.0**
Parents	3	2068.9	5.5**
Hybrids	5	2929.9	7.8**
General	3	3660.9	9.7**
Specific	2	1833.4	4.9*
Error	50	376.8	
Total	65		

* Indicates significance at 0.05.
** Indicates significance at 0.01.

The random group, averaging 1,314 eggs per day, laid more eggs than the D × H F_1, was about equal to the D × F F_1, and was inferior to the other four F_1's. The productivity of the random group exceeded all four of the parental inbreds.

An analysis of the contributions to the observed variation in oviposition rate and honey yield is presented in table 2.

There was a significant difference between the productivities of the 11 groups. The analysis further breaks down the 10 degrees of freedom associated with groups, into some of the contributing causes to the group differences. For oviposition rate, the random and parents, taken as a group, are significantly less productive than the hybrids; the random group was significantly more productive than the parental inbreds; and, the parents differ from each other in relative productivity. The hybrids differed from each other in productivity, with both general and specific combining abilities making significant contributions to the total measured variance.

The oviposition rates given above were measures of the queen mothers' genotypes. The honey yields were measures of productivity associated with the worker progeny tofhe colonies. The mean honey yield for group and replicate are presented in table 1.

The yields associated with progeny of random mated inbred females range from a low of 154 pounds for line D, to a high of 199 pounds for line H. The average for the four inbred female lines was 177 pounds.

Progeny of the random F_1 queens gave yield figures ranging from 199 to 256 pounds. The averages given were all as high as, or higher than, the yields observed for the progeny of random mating inbred females. The average for all of the random mating F_1 queens was 219 pounds, an increase of 42 pounds of honey over the average of the hives led by an inbred queen.

The random group, averaging 207 pounds of honey per colony, was about midway between the inbred and F_1 groups. The random group exceeded all four parental groups, but was itself exceeded by four of the six F_1 groups.

A variance analysis based on the honey yield table of means is presented in table 2. There was a significant difference between the honey gathering capacity of the 11 groups. In the breakdown of the 10 degrees of freedom associated with groups, the hybrids were shown to be significantly greater in yield than the randoms. The randoms, on the other hand, were significantly better yielders than the average of the inbred parental progeny. The inbred parents were shown to be different from each other in their ability to transmit genes associated with honey yield.

The hybrids differed from each other in yield. The sum of squares associated with the hybrids was broken down into that portion due to general and specific combining ability.

DISCUSSION AND INTERPRETATION

Replication differences

The data from the six replicated apiaries indicated comparatively little difference between apiaries for oviposition rate, and a significant difference between replications for honey yield. This could have been predicted for the organism concerned. Honey bee colonies of equal worker strength would be expected to vary in yield if the apiaries were widely separated.

Those factors affecting oviposition rate—such as length of daylight hours, high and low temperatures, early nectar and pollen availability, climatic effects—would be expected to be fairly equal in their effects over the 25 mile range of these apiaries. On the other hand, factors affecting yield—such as concentration of nectar-bearing plants, variation in soil types, and variation in soil fertility—would be expected to vary to a considerable extent over the same 25 mile range.

Parent-progeny comparisons

Table 3 summarizes the performance of the inbreds and their F_1 crosses for both yield and oviposition rate data. It is apparent from a study of these data that the inbred performance was a poor criterion by which to judge the potential performance of F_1 crosses. In the oviposition data, the poorest performing inbred, J, with an 876 egg per day average, was a parent in F_1 combinations yielding the highest average performance, 1,486 eggs per day. The highest producing inbred, H, with a 1,140 eggs per day average, was a parent of the F_1's giving the next to the lowest average performance, 1,391 eggs per day. Inbreds J and D, the two lowest performing inbreds

TABLE 3

Inbred and crossbred productivities

Oviposition rate					Honey yield				
	D	F	H	J		D	F	H	J
D	922	1324	1208	1534	D	154	200	199	216
F		957	1461	1426	F		181	234	212
H			1140	1504	H			198	256
J				876	J				174

Average for inbreds based on crossbred performance

D	F	H	J	D	F	H	J
1355	1404	1391	1486	205	215	229	228

for egg production, produced the F_1 with the highest productivity for the same characteristic.

Inbred H, the highest performing inbred for yield, was a parent of the F_1 having the highest performance. Inbred J, on the other hand, the next to the lowest in inbred performance, was in hybrid combinations that almost equalled the performance of the F_1's involving the H line.

The comparisons of these means merely state in a different form what may be shown with regard to general and specific combining ability. These components of variance may be derived in a variety of ways, SPRAGUE and TATUM (1942), etc. A recent summary of ROJAS (1951) presents an easy approach to the computation of these values.

$$g_i = \text{additive effect for the } i\text{th line}$$
$$= \frac{1}{r(a-2)} Y_{i..} - \frac{2G}{ra(a-2)}$$

Where, Y_i equals the total of all crosses involving line i,
G equals the total for all six crosses over all replications,
r equals the number of replications,
and a equals the number of lines involved.

The effects of specific combining ability for particular crosses were computed using the following formula:

$s_{ij} = y_{ij.} - y... - g_i - g_j$
= mean value of the ij cross, minus the overall mean of experiment, minus the additive effects of i, minus the additive effects of j.

The calculated values for general and specific combining abilities for both honey yield and oviposition rate are presented in table 4.

For oviposition data, table 4 ranks the inbreds with regard to high egg laying general combining ability in the following descending order: J, F, H, and D. Of the four lines, only inbred J exhibits high general combining ability for high egg laying. These

TABLE 4

Line contributions for honey yield and oviposition rate general and specific combining abilities

General combining ability	Yield	Oviposition	Specific combining ability	Yield	Oviposition
			JH	+ 8.5	+ 4.5
D	−22.0	− 81.2	JD	+ 5.7	+88.0
J	+12.8	+117.8	JF	−14.2	−92.3
H	+15.2	−27.7	HF	+ 5.7	+88.0
F	− 6.0	−8.9	HD	−14.2	−92.6
			DF	+ 8.5	+ 4.5

results are a parallel of the observations presented in table 3. It must be remembered, however, that the general combining ability given here was computed on a small amount of data. Only four inbred lines were used in this study and comparisons for general combining ability were based entirely upon the performance of these four lines in cross combinations.

In spite of these limitations, these comparisons are of interest because of the information they give on particular types of gene action. General combining ability, taken as a measure of additive gene action, is shown to vary widely between the four inbred lines studied. Specific combining ability, measuring specific gene combinations leading to the expression of dominance, over-dominance, or epistacy in the phenotypes, is shown to be of importance in the determination of four of the six F_1's studied for oviposition rate. In table 2 general combining ability for the 4 inbreds is shown to be slightly less important to egg yield than specific interactions of the parental genotypes. For honey yield, however, general combining ability is twice as important, 3660.9 to 1833.4, as specific combining ability. In both cases the mean squares indicate that genes having both additive and dominance effects are significant to yield.

Table 4 ranks the inbreds with regard to high yield general combining ability in the following descending order: H, J, F, and D. These results parallel the means given in table 3. Two inbreds, H and J, were about equal in their contributions of additive gene effects for yield. Specific effects for yield were more uniformly distributed over the six F_1 genotypes than for the oviposition data. For the particular data, the high yield performance of the random mated J × H F_1 was accounted for by high additive gene effects contributed by the two inbred parents.

F_1 versus higher parent

Using the original data, each hybrid was tested against its higher parent in a series of 12 analyses of variance. The productivity of one of the hybrids, (D × H), was no better than its higher parent, H, in the oviposition rate comparisons. All of the other F_1's exceeded their better parents in oviposition rate. Expressed as a percentage of the higher parent, these productivities ranged from 128 percent to 166 percent, with an average productivity increase over all F_1's of 35.5 percent.

Honey yield comparisons showed that two of the random mating F_1's could not be considered statistically different from their random mating higher parents. The remaining four random mating F_1's outproduced their higher parents. Expressed as a

percentage of the higher parent, these yields ranged from 117 percent to 129 percent. The average yield of all six F_1's was 115 percent of the highest yielding parent in each cross.

Hybrids versus random

The comparison of the hybrids with the random stock available in the country is interesting from the standpoint of the advisability of the use of inbreeding and hybridization as an economic breeding tool in the honey bee.

Table 1 indicated that the mean oviposition rate of the hybrid queens was 1,409 eggs per day, while that of the random check stock was 1,314 eggs per day. Expressed in terms of the random stock performance, the average of the hybrids was 107.2 percent of the random stock. One hybrid was less than the random stock, one was equal, and the other four exceeded the random stock mean. The two better oviposition rate hybrids, (J × H) and (J × D), exhibited productivities of 114.4 and 116.7 percent of the random stock.

Table 1 showed a mean honey yield of 207 pounds for the random stock colonies, while the progeny of the random mated F_1's yielded an average of 220 pounds per colony. Expressed in terms of the random stock, the average of the hybrids was 106.2 percent. The better yielding hybrid, (J × H), produced at the rate of 123.7 percent of the random stock.

Significance of environmental and inheritance effects on hybrid phenotypes

Recognition of factor combinations which will be low, medium or high in performance under given environments is desirable in any attempt to utilize hybrid vigor. The yield data of table 1 on hybrids and their parental strains as observed in six locations were compared for this purpose. The variance, correlation and regression analyses are found in tables 5 and 6.

The first analysis showed that the hybrid honey yields were affected strongly by the type of cross and particularly by the location of the hive. Adjusting the honey yield of the hive for the estimate of the egg laying capacity of the queen heading the hive reduced the significance of the type of cross somewhat while the effect of location on yield, although reduced in value, still remains highly significant. High egg producing characteristics in the queen were desirable although they did not supply all that was necessary to high honey yield. Good honey gathering ability in each worker was important. The effects of location indicated that adequate quantities of honey plants were necessary to any type of bees.

The second analysis in table 5 considered the data of the inbred parental strains. The conclusions were like those for the hybrids but good honey environment came in for greater emphasis. This was due presumably to the lesser activity of the inbreds.

Tables 5 and 6 present the analyses for honey and egg laying characteristics in the parental inbreds and hybrids. There were important hybrid differences in egg production even after adjustment for mid-parent egg production. This supports the conclusions from table 2 that the contributions of the parents for inherited egg production in the hybrids were only in part additive in type. Dominance and interactions of gene pairs contributed to the egg laying capacity of these bees.

TABLE 5

Variances of hybrid and mid-parent egg productions and honey yields in different crosses, locations and within crosses and locations

Sources of variation	Variance analysis			
	d.f.	Mean square	d.f.	Mean square
Hybrid honey yield	Hybrid honey yield adjusted for hybrid egg production			
Among hybrids	5	2936**	5	1509*
Among locations	5	7424**	5	5052**
Within	25	717	24	532
Mid-parent honey yield	Mid-parent honey yield adjusted for mid-parent eggs produced			
Among hybrids	5	621*	5	366
Among locations	5	10774**	5	10238**
Within	25	203	24	194
Hybrid honey yield	Hybrid honey yield adjusted for mid-parent honey yield			
Among hybrids	5	2936**	5	2372*
Among locations	5	7424**	5	4217**
Within	25	717	24	747
Hybrid eggs produced	Hybrid eggs produced adjusted for mid-parent eggs produced			
Among hybrids	5	90247**	5	92663**
Among locations	5	37428	5	37382
Within	25	17122	24	17307

TABLE 6

Correlations and regressions of hybrid honey yield and egg production as related within themselves, in different locations and to mid-parent yields

Sources of variation	Regression equations	Correlation
A. Crosses	Hybrid honey yield = 41 + 0.13 F_1 egg yield	0.705
A. Locations	Hybrid honey yield = −207 + 0.30 F_1 egg yield	0.681
Within	Hybrid honey yield = 78 + 0.10 F_1 egg yield	0.504
A. Crosses	Hybrid honey yield = −2462 + 1.51 mid-parent honey yield	0.694
A. Locations	Hybrid honey yield = −790 + 0.57 mid-parent honey yield	0.685
Within	Hybrid honey yield = 178 + 0.02 mid-parent honey yield	0.013
A. Crosses	Hybrid egg yield = 1948 − 0.59 mid-parent egg yield	0.304
A. Locations	Hybrid egg yield = 1305 + 0.11 mid-parent egg yield	0.083
Within	Hybrid egg yield = 1190 + 0.23 mid-parent egg yield	0.172

Variances in hybrid honey yields were noticeably reduced for both among hybrids and among locations when adjustment was made for hybrid egg yield on mid-parent performance. The among hybrid reduction in variance may be attributed to genes which were largely additive in their actions on the phenotypes. Analysis of the data in table 2 bears out this conclusion. The reduction in the location variance may be interpreted as due to the hybrid honey yields being affected by many of the same factors as those important to the parental inbreds. Adjustment for mid-parent honey yield consequently reduces the among locations variance. The hybrid honey yield variance was but partially controlled by the mid-parental performance or by the performance in a given location.

The correlations and regressions of hybrid yields with the different attributes are presented in table 6.

The first set of regressions and correlations relate worker collected honey yields to the estimates of egg yields by the queens. The correlations show that among strains of hybrids there is a high correlation between honey stored and the egg laying capacity of the queen. This relation is somewhat less among locations. It is even less within strains and locations. In terms of the method by which the egg production of the hybrid queen was estimated among crosses, an increase of 10 eggs would indicate an increase of 1.3 pounds of honey over the season. For among the hybrid queens in different locations an increase of 10 eggs would increase the honey yield 3.0 pounds. The significance of a good environment for honey gathering was again evident.

The second set of regressions and correlations measures the effects contributed by the mid-parent to the productivity of the hybrid. The correlation between hybrid honey yields and the mid parent honey yields among crosses is rather high. This indicates that in selecting inbred strains to cross the ones chosen should have high honey yields if the hybrids are to be superior. The relation of the hybrid and mid-parent honey yields among locations indicates that the effect of the availability of food supplies was as great as that of inheritance in influencing honey yields. The mean squares and correlations of hybrids and mid-parents within crosses and location were low indicating that most of the information on hybrid performance had been gained when account was taken of inbred mid parents and location differences.

The third set of regressions and correlations measures the effects of the mid-parent egg yields on the egg yields of the hybrids. These correlations are rather low. The highest correlation is negative. This would indicate that lower inbred parent egg productions result in higher egg yields of the F_1 queens. Such a relation would be expected if increasing homozygosity tends to lower performance. The genotypes of the homozygous strains would tend to differ. If the degree of homozygosity is related to the lowering of the yields then the crosses of the most homozygous but genotypically different strains should tend to have the highest yields.

These results on bees have significant parallels in other species. In untested inbred corn material, SPRAGUE and TATUM (1942) showed that general combining ability was of greater importance to yield than was specific combining ability. This was taken as an indication that with untested material additive effects were more important than epistatic and dominance effects. In tested lines, however, it was shown that specific combining ability was about ten percent greater than general combining

ability. ROJAS (1951), in an analysis of combining ability in corn, showed that the average general and specific combining abilities were of about the same importance, but that interactions of specific combining ability with districts and years were of considerably greater magnitude than the same interactions for general combining ability.

The genetic mechanism of heterosis has been investigated experimentally in *Drosophila melanogaster*. STRAUS (1942) showed that the factors responsible for heterosis were located on all chromosomes and that the relationship between vigor and chromosomal heterozygosis was linear. GOWEN and JOHNSON (1946) were able to show racial differences in egg production with higher egg productions for the hybrid Drosophila studied. Heterotic increase in egg production was shown to be associated with greater daily egg-laying rates. A later study by GOWEN, *et al.* (1946) indicated agreement with the hypothesis that hybrid vigor was caused by differences in gene constitution and genic interaction between alleles rather than by some unknown physiological stimulation.

LOH (1949) found no trend in the correlation coefficients between successive generation tests on the same top crosses in Drosophila. The top cross tests contributed little information of value for predicting subsequent performance in later generations. Correlation coefficients between general combining ability values of three different generations were found to be negative and small. In a synthesis and analysis of Drosophila data relating to heterosis, GOWEN (1952) showed that unselected parental inbreds gave F_1 progeny in which general combining ability was more important to productivity than was specific combining ability.

SUMMARY

Two honey bee characteristics, oviposition rate and honey yield, were studied in four inbred lines, their six non-reciprocal F_1's, and a random check stock in 6 different locations.

Heterotic effects were demonstrated for both oviposition rate and honey yield. These effects were such that five of the six hybrid queen groups exceeded their higher parent in oviposition rate, and four of the groups headed by hybrid queens exceeded their higher parent for honey yield.

Expressed as a percentage of the higher parent, the egg productivities of the hybrid queens ranged from 128 percent to 166 percent, with an average productivity increase of 35.5 percent.

Hybrid queen honey yields ranged from 100 percent to 129 percent of the higher parent, with an average yield over the higher inbred parent of 15 percent.

The oviposition rate of the average hybrid queen was 107.2 percent that of queens selected at random from the stock distributed throughout the United States. The two better hybrids exhibited productivities of 114.4 and 116.7 percent of the random stock.

Under the conditions of this experiment, inbreds were shown to exhibit both general and specific combining ability for oviposition rate and honey yield. Gene action leading to general combining ability was of greater importance for honey yield than for egg laying ability. Gene combinations having additive effects and those having

dominance and epistatic effects were of importance in determining egg laying and honey gathering ability. Location differences in nectar plants etc. were shown to be important to the yield performance of the different genotypes.

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CONTENTS OF PRECEDING ISSUES

SEPTEMBER, 1955

Abstracts of Papers Presented at the 1955 Meetings of the Genetics Society of America.

WHITE, M. J. D., and F. H. W. MORLEY, Effects of pericentric rearrangements on recombination in grasshopper chromosomes.

NADEL, ELI M., JOSEPH GREENBERG, GEORGE E. JAY, and G. ROBERT COATNEY, Backcross studies on the genetics of resistance to malaria in mice.

ALLEN, SALLY LYMAN, Linkage relations of the genes histocompatibility-2 and fused tail, brachyury and kinky tail in the mouse, as determined by tumor transplantation.

PRICE, SAM, Irradiation and interspecific hybridization in Secale.

NANNEY, D. L., P. A. CAUGHEY, and A. TEFANKJIAN, The genetic control of mating type potentialities in *Tetrahymena pyriformis*.

KEMPTHORNE, OSCAR, The correlations between relatives in inbred populations.

OSTER, I. I., Modification of X-ray mutagenesis in Drosophila. I. Reunion of chromosomes irradiated during spermiogenesis.

EMMERLING, M. H., A comparison of X-ray and ultraviolet effects on chromosomes of *Zea mays*.

JENKINS, J. A., and G. MACKINNEY, Carotenoids of the apricot tomato and its hybrids with yellow and tangerine.

LOCKHART, WILLIAM R., and HAROLD R. GARNER, Genetic mechanisms governing the effect of canavanine on *Neurospora crassa*.

RYAN, FRANCIS J., Spontaneous mutation in non-dividing bacteria.

JUDD, BURKE H., Direct proof of a variegated-type position effect at the white locus in *Drosophila melanogaster*.

ROBERTSON, DONALD S., The genetics of vivipary in maize.

NOVEMBER, 1955

YANOFSKY, CHARLES, and DAVID M. BONNER, Gene interaction in tryptophan synthetase formation.

COHEN, CARL, Blood group factors in the rabbit. II. The inheritance of six factors.

DOBZHANSKY, TH., O. PAVLOVSKY, B. SPASSKY, and N. SPASSKY, Genetics of natural populations. XXIII. Biological role of deleterious recessives in populations of *Drosophila pseudoobscura*.

DOBZHANSKY, TH., and HOWARD LEVENE, Genetics of natural populations. XXIV. Developmental homeostasis in natural populations of *Drosophila pseudoobscura*.

ZIMMERING, STANLEY, A genetic study of segregation in a translocation heterozygote in Drosophila.

JAMES, ALLEN P., and BRENDA LEE-WHITING, Radiation-induced genetic segregations in vegetative cells of diploid yeast.

LEVINE, LOUIS, Genotypic background and heterosis in *Drosophila pseudoobscura*.

BROWN, SPENCER W., and DANIEL ZOHARY, The relationship of chiasmata and crossing over in *Lilium formosanum*.

ZOHARY, DANIEL, Chiasmata in a pericentric inversion in *Zea mays*.

KIMBALL, R. F., and NENITA GAITHER, Behavior of nuclei at conjugation in *Paramecium aurelia*. I. Effect of incomplete chromosome sets and competition between complete and incomplete nuclei.

KØLMARK, G., and N. H. GILES, Comparative studies of monoepoxides as inducers of reverse mutations in Neurospora.

STEPHENS, S. G., Linkage in Upland cotton.

WELSHONS, WILLIAM J., A comparative study of crossing over in attached-X chromosomes of *Drosophila melanogaster*.

MCDONALD, DANIEL J., Segregation of the selective advantage obtained through orthoselection in *Escherichia coli*.

HINTON, CLAUDE W., The behavior of an unstable ring chromosome of *Drosophila melanogaster*.

Index to Volume 40

JANUARY, 1956

RHOADES, M. M., Lewis John Stadler.

OSHIMA, CHOZO, TOKUICHIRO SEKI, and HIRONORI ISHIZAKI, Studies on the mechanism of pattern formation in the elytra of lady beetles.

HANSON, EARL D., Spontaneous mutations affecting the killer character in *Paramecium aurelia*, variety 4.

GERSTEL, D. U., Segregation in new allopolyploids of Gossypium. I. The R_1 locus in certain New World-wild American hexaploids.

BARISH, NATALIE, and ALLEN S. FOX, Immunogenetic studies of pseudoallelism in *Drosophila melanogaster*. II. Antigenic effects of the vermilion pseudoalleles.

LINDSLEY, D. L., G. FANKHAUSER, and R. R. HUMPHREY, Mapping centromeres in the axolotl.

DICKINSON, A. G., and J. L. JINKS, A generalised analysis of diallel crosses.

CHOVNICK, ARTHUR, and ROBERT J. LEFKOWITZ, A phenogenetic study of the lozenge pseudoalleles in *Drosophila melanogaster*. I. Effects on the development of the tarsal claws in homozygotes.

GEISSMAN, T. A., E. H. HINREINER, and E. C. JORGENSEN, Inheritance in the carnation, *Dianthus caryophyllus*. V. The chemistry of flower color variation, II.

LASKOWSKI, WOLFGANG, The genetics of resistance of Saccharomyces to lithium chloride.

CASPARI, ERNST, The inheritance of the difference in the composition of the liver mitochondria between two mouse strains.

STURTEVANT, A. H., A highly specific complementary lethal system in *Drosophila melanogaster*.

STADLER, L. J., and M. H. EMMERLING, Relation of unequal crossing over to the interdependence of R^r elements (P) and (S).

COCKERHAM, C. CLARK, Effects of linkage on the covariances between relatives.

MORSE, M. L., ESTHER M. LEDERBERG, and JOSHUA LEDERBERG, Transduction in *Escherichia coli* K-12.

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